Appendix A

Benefits of DoD Animal Care and Use Programs

Appendix A

Benefits of DoD Animal Care and Use Programs

Alternatives to Animal Research, Breeding Programs (A1, A2, B)

- Development of frog embryos instead of mammals as mutagenesis models
- Development and use of fish instead of mammals as carcinogenesis models
- Development and use of roundworms as a model for testing developmental toxicity
- Establishment of a knockout mouse strain to the study of immunologic tolerance related to organ transplantation

Clinical Medicine (C1)

- Characterization of osteoclasts toward understanding bone tissue repair
- Development of a drug that prevents kidney failure and damage
- Discovery and characterization of a new class of antibiotics from algae for use against antibiotic resistant infection
- Studies of hemorrhage in arterial plaque disruption and arterial thrombosis
- Study of oxygen prevention of blood pressure hemorrhagic blood loss
- Determination of the effectiveness of exogenous surfactant in the treatment of acute lung injury
- Development of a model to study the development of alcoholic liver disease
- Development of a model to study ocular and peripheral neuropathy, and diabetes II
- Study of the effects of nitric oxide treatment on hyperoxia-induced pathology
- Research on the origin and role of vasopressin in hypertension
- Determination that low dose salicylate may protect against hearing loss
- Determination that combined antioxidants can prevent and to some extent, reverse noise-induced hearing loss
- Characterization of an effective medication for treating ocular aspergillosis
- Identification of a simple treatment for cutaneous fungal infections
- Research to develop a highly improved anthrax vaccine for military and civilian use
- Characterized glutamate excitotoxicity, which occurs in head injury and seizures
- Evaluated hereditary influences on smooth airway function
- Characterization of the effect of smoke on lung secretions
- Toward understanding flight physiology, studied microgravity effects on lung ventilation
- Improved standard of care for brown recluse spider bites
- Studies of the effects of estrogen on hemorrhage and the potential differential effects of hemorrhagic shock to women
- Identification of a critical window for treatment of hypertension
- Investigation of therapeutic treatment modalities against blunt trauma

Clinical Surgery (C2)

- Development of a rodent model to study Staphylococcus-induced acute osteomyelitis
- Optimization of bone implantation
- Characterization of more effective biomedical lasers for medical use
- Development of a model of space travel-induced musculoskeletal injuries
- Development of a model for penetrating diaphragmatic injury
- Design of innovative surgical implants for aortic root replacement and corneal repair
- Application of a dry fibrin sealant bandage to prostate removal
- Determination the optimal timing of removal of lead pellets from a joint
- Development of a new and valuable goat model of scoliosis
- Testing a new and promising blood replacement for battlefield use
- Development of better surgical methods for jaw reconstruction and joint ligament repair
- Study of the destabilizing role of magnesium loss in heart failure
- Comparison of vascular responses to balloon injury and/or stenting between young and old animals

Other Clinical Studies (C3)
- Studies to identify the nitric oxide (N_2O) receptors and/or sites of action

Infectious Diseases (M2)
- Study of the effects of mycoplasmas on malignant transformation
- Establishment of colonies of mosquitoes for malaria research
- Development of a field site for testing dengue vaccine candidates
- Development and demonstration of an effective oral vaccine against *Brucella*
- Confirmation that CpG DNA can provide a dose-dependent protection against *Brucella* challenge
- Determination that chloroquine, a major antimalarial, may increase virus infection
- Demonstration that scrub typhus infected mice produce antibodies that kill HIV-1 virus
- Identification of new vectors for leishmaniasis
- Development of a model of genital tract infection by *Neisseria gonorrheae*
- Pathogen analysis of 1,272 samples from patients with central nervous system disease
- Identification of meningitis/encephalitis in patient samples associated with Rift Valley fever and West Nile virus
- Characterization the distribution of arbovirus infection in upper Egypt
- Identification of a new ecotype and vector for scrub typhus
- Design, identification, and evaluation of antimalarial compounds
- Demonstration of an effective vaccine against Brucella melitensis challenge in mice
- Development and testing of schistosomiasis recombinant vaccine candidates
- Development of a vaccine for hantavirus infection
- Determination of mosquito components that induce host resistance
- Characterization of regional, militarily relevant viral disease threats, including new viruses and strains of known viruses
- Demonstration of the superior protection offered by sequentially administering DNA and protein vaccines
- Transgenic mice were used to produce human monoclonal antibodies for Ebola virus
- Development of a murine model of gonococcal genital tract infection by *N. gonorrhoeae*
- Development of rapid diagnostic tests for cariogenic bacteria to address military dental readiness needs
- Development of a rapid assay to identify the presence or absence of malarial pathogens for use in medical threat assessments for military deployments
- Characterization of cellular and inflammatory effects caused by malarial pathogens
- Development of insecticides and larvacides for antimalarial use
- Comparison of meningococcal vaccine immunization routes
- Demonstration that hybridoma clones offer a non-animal alternative to animals for the production of monoclonal meningococcal antibodies
- Development of a vaccine for *Shigella*, an intestinal bacterial pathogen

Medical Chemical Defense (M3)
- Understanding the medical consequences of low-dose chemical warfare agents
- Risk priortization in a chemically contaminated environment and assistance to medical personnel in accurately identifying casualties for treatment
- Performance of a comprehensive toxicological evaluation of novel threat agents the effectiveness of current medical countermeasures against them
- Development of behavioral assessment batteries to assess threat agent effects
- Evaluation of the performance, learning, and memory effects of candidate anticonvulsants

Medical Biological Defense (M4)
- Production and evaluation of a new vaccine for Venezuelan equine encephalitis
- Evaluation of vaccine technologies for use in multiple agent delivery with one vaccination

- Development of a new recombinant anthrax vaccine against inhalation infection
- Testing of *Botulinum* vaccine candidates
- Improvement of diagnostic assays for tularemia, plague, and melioidosis
- Demonstration of an effective Ebola vaccine in mice
- Demonstration of the effectiveness of transcutaneous immunization

Human Systems Technology (M5)
- Determination of bioeffects and safety levels of laser, microwave, and electromagnetic radiation
- Characterization of the molecular physiology of heat injury/stroke/stress
- Characterization of the molecular physiology of hypothermia
- Effect of menstrual cycle hormones on performance
- Impact of Levonorgestrel (Norplant) contraceptive on endurance capacity and exercise
- Investigation of cerebral blood flow and blood pressure as predictors of seizure time related to hyperbaric oxygenation
- The development of potential anti-oxygen toxicity treatments
- Characterization of adult respiratory distress syndrome (ARDS)

Combat Casualty Care (M6)
- Development of microencapsulated antioxidants to preserve skin flaps in injury
- Characterization and prevention of seizures induced by hyperbaric oxygen
- Provision of safe and effective ventilatory support and suctioning in aeromedical evacuation
- General resuscitation strategies and transportation requirements
- Controlling lethal hemorrhagic shock with the development and evaluation of new hemostatic dressings and pharmacologic agents
- Evaluation of skin grafting techniques and the use of dermal replacement tissues
- Decreasing secondary wound infection in orthopedic patients by specially coating external fixator pins
- Characterizing the relationship between injury repair and growth factors
- Development of a drug that is effective in the treatment of traumatic brain injuries
- Development of a prophylatic (preventative) drug to protect against hypoxia/ischemic-reperfusion
- Training of the Forward Surgical Teams in advanced traumatic life support techniques and other expected medical emergencies in combat and non-combat situations
- Understanding the mechanisms of combat casualty wounds or injuries
- Clarifying cellular-molecular changes associated with head injury
- Validation of a new portable noninvasive method for diagnosing head injury
- Demonstration of a fibrin sealant hemostatic dressing in reducing bleeding time and blood loss
- Validation of an animal model for human posttraumatic epilepsy
- Studied the relationship between dietary antioxidants and seizure episode severity
- Study of iron chloride-induced seizures, which can generate permanent posttraumatic epilepsy following shrapnel penetrating head wounds
- Suppression of brain inflammatory response in improving head injury outcome
- Characterization of the neurotoxic side effects of neuroprotective drug candidates
- Clarification of the relationship between arterial pressure and head injury severity

Ionizing Radiation
- Determination of nonandrogenic androstene steroids as novel, effective, and nontoxic radioprotectants
- Evaluation of the use of cytokine combinations following radiation exposure

Appendix B

Journals and Proceedings with DoD Animal Research Publications by Research Category

Appendix B

Journals and Proceedings with DoD Animal Research Publications by Research Category

JOURNALS

Animal Use Adjuncts/Alternatives (A1, A2)
- American Journal of Physiology
- Aquatic Mammals
- Journal of Veterinary Pharmacology and Therapeutics
- Journal of Zoo and Wildlife Medicine
- Marine Mammal Science
- Toxicologic Pathology
- Veterinary Pathology

Clinical Medicine (C1)
- Biology of the Neonate
- Cell and Molecular Biology
- Cell Biology International
- Developmental Brain Research
- Endocrinology
- Infection and Immunology
- International Journal of Development
- Journal of Immunology
- Muscle and Nerve
- Neurogastroenterology and Motility
- Neurotoxicology
- Otolaryngology - Head and Neck Surgery
- Radiation Research

Clinical Surgery (C2)
- American Journal of Critical Care
- Journal of the American College of Cardiology
- American Surgeon
- The Journal of Trauma
- Journal of Bone and Joint Surgery
- Journal of Surgical Research
- Journal of the Society of Laparoendoscopic Surgeons
- Journal of Urology
- Nature Medicine
- Semin Intervent Cardiol
- The American Journal of Cardiology
- The Journal of Orthopaedic Research

Clinical Studies - Other (C3)
- Cerebral Cortex
- Journal of Pharmacology and Experimental Therapeutics

Infectious Disease (M2)
- American Journal of Tropical Medicine and Hygiene
- Antimicrobial Agents and Chemotherapy
- Biophysical Chemistry
- Clinical Infectious Diseases
- European Journal of Immunology
- Expert Opinion on Investigational Drugs
- Experimental Cell Research
- Experimental Parasitology
- Infection and Immunity
- Journal of Infectious Disease
- Journal of Medical Entomology
- Journal of Pharmacological Sciences
- Journal of Virology
- Journal of the American Mosquito Control Association
- Journal of General Virology
- Journal of Immunology
- Journal of the Egyptian Society of Parasitology
- Medical Veterinary Entomology
- Molecular Cell Biology
- Molecular and Biochemical Parasitology
- Proceedings of the National Academy of Sciences
- The Lancet
- Virology

Medical Chemical Defense (M3)
- Archives of Toxicology
- CNS Drug Reviews
- Drug and Chemical Toxicology
- Environmental Toxicology and Pharmacology
- Inhalation Toxicology
- Journal of Applied Toxicology
- Journal of Biomedical Science
- Journal of Toxicology - Cutaneous and Ocular Toxicology
- Mathematical Biosciences
- NIDA Monograph Series
- Pharmacology, Biochemistry & Behavior
- Psychopharmacology
- Skin Research and Technology
- Toxicology Methods

Medical Biological Defense (M4)
- American Journal of Tropical Medicine Hygiene
- Archives of Pathology and Laboratory Medicine
- Biophysics Journal
- Canadian Journal of Microbiology
- Clinical and Diagnostic Laboratory Immunology
- Entomology News
- Expert Opinion on Investigational Drugs
- Infection and Immunity
- International Journal of Biological Macromolecules
- Journal of Pharmacological Sciences
- Journal of Immunology
- Journal of Infectious Diseases
- Journal of Leukocyte Biology
- Journal of Medical Entomology
- Journal of Natural Toxins
- Journal of Toxicology, Toxicology Reviews
- Microbial Infection
- Toxicology
- Vaccine
- Veterinary Pathology
- Virology

Human Systems Technology (M5)
- Bioelectromagnetics
- Biotechnic and Histochemistry
- Journal of Thermal Biology
- Physics in Medicine and Biology

Combat Casualty Care (M6)
- FASEB Journal
- Journal of Burn Care and Rehabilitation
- Nature Medicine
- American Surgeon
- Analytical Chemistry
- Clinical Immunology and Immunopathology
- Current Surgery
- European Journal of Pharmacology
- Journal of Pharmacology and Experimental Therapeutics
- Journal of Trauma
- Military Medicine
- Protein

Ionizing Radiation (M7)
- Aviation, Space, and Environmental Medicine
- Biotechnic and Histochemistry
- Journal of Leukocyte Biology
- Journal of Neurovirology
- Mutagenesis
- Radiation Oncology Investigations
- Radiation Research
- Toxicological Sciences

Medical Studies - Other (M8)
- Drug and Chemical Toxicology
- Journal of Applied Physiology

Non-Medical Research - Physical Protection and Detection (N1, N2)
- Applied Physics
- Applications of Ultrashort Pulse Lasers in Medicine and Biology
- Aviation, Space, and Environmental Medicine
- Bioelectromagnetics
- Biosensors and Bioelectronics
- FASEB Journal
- Health Physics
- Journal of Laser Applications
- Journal of Structural Biology

Non-Medical Research - Other RDT&E (N4)
- Environmental Toxicity and Chemistry
- Journal of Chromatography B: Biomedical Applications
- Journal of Toxicology and Environmental Health
- Toxicology and Applied Pharmacology
- Toxicological Sciences
- Toxicology Methods

PROCEEDINGS

Clinical Medicine (C1)
- American Journal of Respiratory and Critical Care Medicine
- Federation of American Societies for Experimental Biology Journal
- Journal of American Society of Nephrology
- Journal of Burn Care and Rehabilitation
- Journal of Investigative Medicine
- Pediatric Research

Infectious Disease (M2)
- 99th General Meeting of the Society for Microbiology
- American Society of Tropical Medicine and Hygiene
- Journal of Dental Research

Medical Chemical Defense (M3)
- Proceedings of the 1998 Medical Defense Bioscience Review
- Society for Neuroscience Abstracts

Human Systems Technology (M5)
- 21st Annual Meeting of the Bioelectromagnetics Society
- FASEB Journal
- Society for Neuroscience
- Undersea Hyperbaric Medicine

Combat Casualty Care (M6)
- FASEB Journal
- Journal of Burn Care and Rehabilitation
- American Society of Hematology
- Journal of Urology
- Neuroscience Abstracts
- Society of Academic Emergency Medicine

Appendix C

DoD Directive on Animal Use

Department of Defense
DIRECTIVE

April 17, 1995
NUMBER 3216.1

DDR&E

SUBJECT: Use of Laboratory Animals in DoD Programs

References: (a) DoD Directive 3216.1, "Use of Animals in DoD Programs," February 1, 1982 (hereby canceled)
(b) Title 9, Code of Federal Regulations, "Animals and Animal Products," Chapter 1, Subchapter A, "Animal Welfare," Parts 1, 2, and 3
(c) Public Law 101-511, Department of Defense Appropriations Act for Fiscal Year 1991, Section 8019, Title 10 United States Code, Section 2241
(d) Sections 2131 through 2156 of Title 7, United States Code "The Laboratory Animal Welfare Act of 1966," as amended
(e) through (f), see enclosure 1.

A. REISSUANCE AND PURPOSE

1. Reissues reference (a) to update policy governing activities using animals within the Department of Defense.

2. Designates the Secretary of the Army as the DoD Executive Agent to develop and issue Service regulations to implement this Directive.

B. APPLICABILITY

This Directive applies to the Office of the Secretary of Defense, the Military Departments, the Uniformed Services University of the Health Sciences, and the Defense Agencies (hereafter referred to collectively as "DoD Components") that perform or sponsor activities using animals.

C. DEFINITIONS

Terms used in this Directive are defined in enclosure 2.

D. DoD POLICY

1. Federal statutes, regulations, and publications that provide national standards and guidance for the acquisition, transportation, housing, control, maintenance, handling, protection, treatment, care, use, and disposal of animals shall be applicable to all activities using animals. A summary of the applicable documents cited as references is in enclosure 3.

2. Animals shall be legally obtained from suppliers licensed by the U.S. Department of Agriculture (USDA) in accordance with

reference (b) unless specifically exempted from the licensing requirements stated in reference (b).

3. DoD organizations or facilities maintaining animals for use in research, testing or training shall apply for accreditation by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

4. Alternative methods to animal species shall be considered, whenever possible, if such alternatives produce scientifically valid or equivalent results to attain the research testing and training objectives.

5. The purchase or use of dogs, cats, or nonhuman primates in research conducted for developing biological, chemical or nuclear weapons is prohibited.

6. The purchase or use of dogs, cats, or nonhuman primates for inflicting wounds from any type of weapon(s) to conduct training in surgical or other medical treatment procedures is prohibited. (reference (c)).

7. DoD organizations or facilities wishing to hold training programs using animals, such as advanced trauma life support (ATLS) training programs, shall have the training protocol reviewed and approved by a duly constituted Institutional Animal Care and Use Committee (IACUC) in accordance with references (d) and (e) and paragraph D.8. of this Directive to ensure the humane use of animals. DoD organizations or facilities conducting ATLS training that require housing of animals for short periods of time shall ensure adequate care and shall have the animal housing facilities inspected and approved by a veterinarian prior to receipt of the animals.

8. All proposals or protocols for animal experiments or demonstrations in RDT&E, clinical investigation, instructional, or training programs conducted or sponsored by a DoD organization or facility shall be reviewed and approved by a duly constituted IACUC composed of a minimum of five members. There shall be at least one non-scientific member on each IACUC. In addition, there also shall be a member who represents the general community interest and is non-affiliated with the facility sponsoring IACUC. The non-affiliated and the non-scientific membership can be filled by the same person. To ensure community representation at each meeting and inspection, an alternate to the non-affiliated member shall be designated for IACUCs having a single non-affiliated membership. Since the DoD IACUCs perform a Government function in an approval process and do not serve merely as an advisory body, the non-affiliated and the non-scientific member(s) to DoD IACUCs shall either be a Federal

employee, with demonstrated commitment to the community or a consultant consistent with the requirements established by reference (f).

9. A headquarters-level administrative review shall be conducted for proposals involving the use of non-human primates conducted or sponsored by subordinate activities of the DoD Component for conformance with all applicable Federal regulations and policies. A DoD component may delegate this responsibility to another DoD component for purposes of efficiency and consolidation of functional offices.

10. The DoD Components shall coordinate and cooperate in the transfer of Government-owned nonhuman primates between facilities to maximize conservation and proper utilization.

11. Proposals intending to use chimpanzees must be further reviewed and approved by the Interagency Animal Model Committee, which coordinates national priorities for research utilization of this species.

12. The DoD components that sponsor animal based research, testing, and training under a DoD grant or contract shall ensure that:

a. all extramural research proposals using live animals shall be administratively reviewed by a DoD veterinarian trained or experienced in laboratory animal science and medicine before grant or contract award.

b. the most recent USDA inspection reports are provided or obtained for the facility under consideration for a research contract or grant using animals, and that during the term of the award, the most recent USDA inspection reports be reviewed on an annual basis.

c. a DoD veterinarian trained or experienced in laboratory animal science and medicine shall conduct an initial site visit to evaluate animal care and use programs at contracted facilities conducting DoD-sponsored research using non-human primates, marine mammals, dogs, cats, or proposals deemed to warrant review. The initial site visit shall occur within 6 months of when the facility has taken delivery of the animals under DoD contract or grant award. Any facility receiving a DoD-funded grant or contract for animal based research shall notify the DoD component sponsor and shall have a site inspection within 30 days of notification of loss of AAALAC accreditation for cause, or notification that the facility is under USDA investigation. Site inspections for cause shall evaluate and

ensure the adequacy of animal care and use in DoD-sponsored programs, and provide recommendations to the sponsoring DoD component about continued funding support of the research.

13. In the case of differences between the standards of care and use of animals as cited in enclosure 3, the most stringent standard shall apply.

14. Activities covered by this Directive that are performed or sponsored in foreign countries shall be conducted in accordance with applicable U.S. statutory requirements, and regulations and standards of the host country. If differences exist between U.S. and host country regulations or standards, unless prohibited by the host country, the more stringent standard shall apply.

15. While not specifically addressed in this Directive, ceremonial, recreational, and working animals, such as military working dogs, shall be treated in a humane manner.

16. Personnel with complaints of violation of this directive shall report such violations to either of the following members of the organization or facility: IACUC chairperson, attending veterinarian, the facility Commander, or Inspector General. The IACUC shall review and, if warranted, investigate all reports of complaints of animal use or noncompliance with 7 U.S.C. 2131-2 of reference (d), applicable Directives, and regulations.

E. RESPONSIBILITIES

1. The Director, Defense Research and Engineering (under the Under Secretary of Defense for Acquisition and Technology) or designee shall:

a. Issue policy and procedural guidance concerning animal use consistent with all applicable Federal regulations and policies.

b. Designate a DoD representative to the Interagency Research Animal Committee who is a veterinarian of appropriate rank or grade and experience, and preferably also a diplomate of the American College of Laboratory Animal Medicine.

c. Establish the Joint Technical Working Group (JTWG) to act as the central advisory committee to the Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee on all matters on the care and use of animals for research, testing, clinical investigation, or training within the Department of Defense. The co-chairpersons of the ASBREM Committee shall designate the chairperson of JTWG.

2. The Heads of the DoD Components shall:

a. Establish appropriate mechanisms to monitor compliance with this Directive and applicable Federal statutes and regulations.

b. Establish offices or facilities that shall serve as reviewing or approving authorities of animal use proposals from subordinate activities and extramural facilities proposing research under contract or grant.

c. Provide members to JTWG as required.

d. Designate the appropriate office(s) within the DoD Component that shall perform the headquarters level administrative review of proposals requiring the use of non-human primates and shall serve as the office where exemptions under paragraph D.2. above may be approved.

e. Support, and as necessary, ensure the development of animal care and use training programs for researchers and members of the IACUC, and certification programs for all personnel involved in the care, use, and treatment of animals.

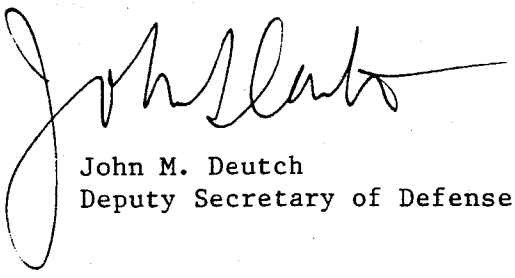
3. The Secretary of the Army shall:

a. As Executive Agent, develop and issue, in consultation with the other DoD Components, joint Service regulations to implement this Directive.

b. Designate the Commander, U.S. Army Veterinary Command/Director, DoD Veterinary Services Activity, a Field Operating Agency of the Army, Office of the Surgeon General who shall serve as a consultant to the Assistant Secretary of Defense for Health Affairs and the Director, Defense Research and Engineering for technical and professional matters related to this Directive.

F. EFFECTIVE DATE

This Directive is effective immediately.



John M. Deutch
Deputy Secretary of Defense

Enclosures - 3
1. References
2. Definitions
3. Guidance Documents

(e) National Institutes of Health (NIH) Publication No. 86-23, "Guide for the Care and Use of Laboratory Animals", United States Department of Health and Human Services, National Institutes of Health, Revised 1985.
(f) Title 5, United States Code, Section 3109.

DEFINITION OF TERMS

1. Animal. - Any dog, cat, non-human primate, guinea pig, hamster, rabbit or any other live vertebrate animal, which is being used or is intended for use for research, training, testing, or experimentation purposes. For this Directive, it includes birds, rats of the genus Rattus and mice of the genus Mus bred for use in research, training, testing or experimentation purposes. The term excludes animals used for ceremonial or recreational purposes, military working animals, and animals intended for use as livestock and poultry as food or fiber; or, livestock or poultry used or intended for use for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber.

2. Clinical Investigation. - All activities directed towards clinical research conducted principally within medical treatment facilities. The Clinical Investigations program is part of the Defense Health Program of the Assistant Secretary of Defense (Health Affairs) and is supported by Major Force Program 8 (MFP-8) funds.

3. Instructional Program. - All educational and training activities, except training of ceremonial and recreational animals and training associated with military working animals or survival skills training.

4. Research, Development, Test, and Evaluation. - All activities which form the RDT&E program of the Director, Defense Research and Engineering (DDR&E) and are supported by Major Force Program 6 (MFP-6) funds.

5. Alternatives. - Any system or method that covers one or more of the following: replacing or reducing the number of laboratory animals required for an investigation by computer simulation, cell culture techniques, etc; or, refining an existing procedure or technique to minimize the level of stress endured by the animal.

6. DoD Sponsored Programs. - All proposals or de signs for animal experiments or demonstration in RDT&E, clinical investigation, or instructional programs conducted or funded by grant, award, loan, contract, or cooperative research and development agreement (CRADA).

ADDITIONAL FEDERAL STATUTES, REGULATIONS, AND GUIDELINES ON THE USE OF ANIMALS

The following documents provide national standards and guidance for the protection, treatment and use of animals:

a. **Animal Welfare Act** (Title 7, United States Code, Sections 2131-2158, as amended, and Title 9, Code of Federal Regulations, Parts 1-4, implementing rules and regulations). Administered by Regulatory Enforcement and Animal Care (REAC), Animal and Plant Health Inspection Service (APHIS) of the Department of Agriculture. Requires licensing of dealers, identification of animals, maintenance of records, submission of reports, establishment of an Institutional Animal Care and Use Committee (IACUC), and compliance with standards for the humane handling, care, treatment, and transportation of animals by dealers and research facilities.

b. **Endangered Species Act of 1973** (Title 16, United States Code, Sections 1531-1543, as amended, and Title 50, Code of Federal Regulations, Parts 10-14 and 217-227, implementing rules and regulations). Provides a program under the U.S. Fish and Wildlife Service, Department of Interior, for conserving threatened and endangered species. Requires import/export permits, maintenance of records, and submission of reports on the care and handling of endangered, threatened, and conserved species.

c. **Marine Mammal Protection Act** (Title 16, United States Code, Sections 1361-1384, as amended, and Title 50, Code of Federal Regulations, Parts 10-14 and 216-227, implementing rules and regulations). Provides a program under the Departments of Commerce (National Marine Fisheries Service) and Interior (U.S. Fish and Wildlife Service) for the protection of marine mammals and marine mammal products. Requires acquisition permits, maintenance of records, submission of reports, and inspections on the care and handling of marine mammals.

d. **Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)** (TIAS 8249, as amended, and Title 50, Code of Federal Regulations, Part 23, implementing rules and regulations). CITES is a treaty involving 106 signatory nations administered in the United States by the Fish and Wildlife Service of the Department of the Interior. CITES regulates the import and export of imperiled species covered by the treaty but imposes no restrictions or control on interstate shipments.

e. **Lacey Act** (Title 18, United States Code, Section 42, as

amended, and Title 50, Code of Federal Regulations, Part 16 and Subpart B, implementing rules and regulations). A program under the U.S. Fish and Wildlife Service, Department of the Interior. Prohibits the importation of certain wild animals or their eggs if the Secretary of the Interior determines that they are injurious to humans, the interest of agriculture, or other specified national interests.

f. **Guide for the Care and Use of Laboratory Animals.** Public Health Service, National Institutes of Health, NIH Publication No. 86-23, Revised. Provides guidelines for institutional policies, husbandry, requirements, veterinary care, and physical plant requirements for programs involving the care and use of laboratory animals.

g. **Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.** Published by the Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, 309 West Clark Street, Champaign, IL 61820, March 1988. Provides guidelines for the care and use of the major agricultural animal species in the United States in research and teaching.

Appendix D

DoD Policy for Compliance with Federal Regulations and DoD Directives for the Care and Use of Laboratory Animals in DoD-Sponsored Programs

OFFICE OF THE SECRETARY OF DEFENSE

WASHINGTON, D.C. 20301

10 APR 1995

MEMORANDUM FOR ASSISTANT SECRETARY OF THE ARMY (M&RA)
ASSISTANT SECRETARY OF THE ARMY (RDA)
ASSISTANT SECRETARY OF THE NAVY (M&RA)
ASSISTANT SECRETARY OF THE NAVY (RDA)
ASSISTANT SECRETARY OF THE AIR FORCE (MRAI&E)
ASSISTANT SECRETARY OF THE AIR FORCE (SAF/AQ)
PRESIDENT, UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
DIRECTOR, DEFENSE NUCLEAR AGENCY
DIRECTOR, ADVANCED RESEARCH PROJECTS AGENCY

SUBJECT: Department of Defense (DoD) Policy for Compliance with Federal Regulations and DoD Directives for the Care and Use of Laboratory Animals in DoD-Sponsored Programs

References:

(a) Title 7, United States Code, Sections 2131-2156, The Laboratory Animal Welfare Act of 1966, PL 89-544, as amended PL 94-279, 1976, and PL 99-198, 1985.

(b) Review of the Use of Animals in the Department of Defense Medical Research Facilities, Inspector General Department of Defense, February 1994.

(c) Review of the Use of Animals in Department of Defense Contract Research Facilities, Inspector General Department of Defense, August 1994.

Definition:

(a) Animal means any dog, cat, non-human primate, or any other live vertebrate animal which is being used or is intended for use for research, training, testing, or experimentation purposes. For this Policy Guidance, it includes birds, rats of the genus Rattus and mice of the genus Mus bred for use in research, training, testing or experimentation purposes. The term excludes animals used for ceremonial or recreational purposes, military working animals, and animals intended for use as livestock and poultry as food or fiber; or, livestock or poultry used or intended for use for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber.

(b) DoD-Sponsored programs means any study, proposal, or design for animal experimentation or demonstration in Research Development, Test, and Evaluation (RDT&E), clinical investigation, or instructional program conducted or funded by grant, award, loan, contract, or cooperative research and development agreement (CRADA).

Reference (a) has been accepted by the Department of Defense (DoD) in the development of DoD Directives and policy guidance. References (b) and (c) contain recommendations which have been endorsed by the Department. The purpose of this policy memorandum is to implement the recommendations contained in references (b) and (c).

DoD components that utilize animals in DoD-supported programs shall be aware of the attached DoD Directive 3216.1, "Use of Laboratory Animals in DoD Programs," appended as attachment (1). It is currently pending signature and will supersede the current DoD Directive 3216.1 dated February 1, 1982. Additional policy guidance is as follows:

a) In DoD component facilities conducting animal-based programs, an alternate to the non-affiliated member of the Institutional Animal Care and Use Committee (IACUC) shall be designated for IACUCs having a single non-affiliated member. The non-affiliated member(s) or alternates must receive a minimum of eight hours training. At least four hours of the training shall address the regulatory responsibilities and proper techniques on animal protocol review processes. An additional minimum of four hours of training will address humane care and ethics issues dealing with animal use. All DoD Components conducting animal use programs as defined shall have training programs for non-affiliated IACUC members in place by 1 October 1995.

b) All DoD component facilities maintaining animals used in research, testing, or training shall apply for accreditation by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). The Office of the Director, Environmental and Life Sciences, Pentagon Room 3D129, Washington, D.C. 20301-3030 is the central point of contact to maintain cognizance over the application or continuation of AAALAC accreditation. All DoD facilities shall furnish copies of AAALAC accreditation status to that office. Absence of accreditation shall be explained with a plan of action and milestones to obtain accreditation.

The following recommendations from the DoD Inspector General have been adopted as policy and shall be fully implemented by DoD Components which use animals in DoD-sponsored programs.

a) The DoD standard protocol format appended as attachment (2) shall be implemented by 1 October 1995. All intramural protocols involving animal use submitted after 1 October 1995 shall use the standard format. Extramural contractor proposal submissions need not use the standard format; however, the contractor shall provide all pertinent information contained in the standardized protocol format.

b) All DoD component facilities that utilize animals in research, testing and training shall implement the DoD standardized semi-annual program review checklist appended as attachment (3) immediately. Accompanying the checklist is a detailed outline of program review as contained in the NIH Guide for the Care and Use of Laboratory Animals. The Guide is the primary reference which is used by AAALAC in the accreditation process. The checklist shall be completed as a part of the semiannual IACUC program and facility review process. The semi-annual IACUC reports shall contain a copy of the checklist or indicate that the checklist was used as the basis of the program and facility review. A majority of members of the IACUC shall sign the report and include a statement indicating the presence or absence of minority opinions.

c) Commanders, and Directors of DoD component facilities shall support and, as necessary, develop animal care and use training programs for personnel associated with animal use programs, and encourage certification for all personnel involved in the care, use and treatment of laboratory animals.

As of 1 October 1995, DoD components shall report all animal-based protocols in the required format redacted for public release to the Defense Technical Information Center (DTIC). Selected fields of the DTIC report will be made accessible to the public through the INTERNET.

Edward D. Martin
Principal Deputy,
Assistant Secretary of
Defense (Health Affairs)

Joseph V. Osterman
Director, Environmental
and Life Sciences

Attachments:

(1) Pending DoD Directive 3216.1
(2) Standard Protocol Format
(3) Standard Semi-annual Checklist

Appendix E

DoD Standard IACUC Protocol Format Instructions

ALL DOD ANIMAL USE PROTOCOLS MUST UTILIZE THIS DOD STANDARDIZED FORMAT. This protocol format only includes those requirements of the Animals Welfare Act, American Association for the Accreditation of Laboratory Animal Care, Federal Regulations, DoD Directives and DoD Policy relating to animal use. Any requirements that are specific to a given Service, Command, or locale (such as all budgeting information, local coordinating requirements, specific scientific review requirements etc.) should be added by each organization in front or behind this standardized format. Adding some information within the format is acceptable to meet local needs as long as the standard format is maintained. In other words, all of the labelled paragraphs and subparagraphs should remain in the same relative order with the added information being similar or complementary to the information requested. It is important to note that this standardized protocol format does not in any way prohibit local organizations from using any (or all) of their current animal use protocol. It does mandate that all of the information required in this DoD standardized format be answered as a part of the organization's animal use protocol in the order listed in this format.

****

THIS DOCUMENT IS INTENDED TO BE AN AID IN THE PREPARATION OF A DOD ANIMAL USE PROPOSAL. IT IS A COMPANION DOCUMENT TO AN IDENTICAL PROTOCOL FORMAT OR TEMPLATE THAT DOES NOT HAVE THE WRITTEN EXPLANATION FOR INDIVIDUAL PARAGRAPHS. THEY ARE DESIGNED TO BE USED ON A WORD PROCESSING PROGRAM, i.e., WordPerfect, WordStar, MicrosoftWord, WordPerfect for Macintosh, etc., SO THAT YOU ARE NOT LIMITED BY THE SPACE PROVIDED, AND SUGGESTED CHANGES OR MODIFICATIONS CAN BE QUICKLY AND EASILY MADE. USING A WORD PROCESSOR MAKES THIS FORMAT A "FILL-IN-THE-BLANKS" EXERCISE. THE EXPLANATIONS OR INSTRUCTIONS MAY BE BLOCKED OUT AND DELETED IF IT IS MORE CONVENIENT TO USE THIS FORM RATHER THAN THE OUTLINE AVAILABLE WITHOUT THE EXPLANATIONS. SPECIFIC RESPONSES REQUESTED IN THE FORMAT ARE A RESULT OF THE REQUIREMENTS OF THE ANIMAL WELFARE ACT (AWA), DOD REGULATIONS, OR ANIMAL WELFARE GUIDELINES. EACH PARAGRAPH SHOULD HAVE A RESPONSE. PORTIONS OF THE PROTOCOL FORMAT THAT ARE NOT APPLICABLE TO YOUR PARTICULAR PROTOCOL, i.e., NO SURGERY OR NO PROLONGED RESTRAINT, SHOULD BE MARKED N\A. IF SOPs OR OTHER DOCUMENTS ARE READILY AVAILABLE TO THE IACUC, THEY MAY BE REFERENCED TO ASSIST IN THE DESCRIPTION OF SPECIFIC PROCEDURES. IT IS CRITICAL THAT ONLY ANIMAL STUDIES OR PROCEDURES DOCUMENTED IN AN APPROVED PROTOCOL ARE PERFORMED IN THE ORGANIZATION. ADDITIONALLY, P.I.s OR OTHER ANIMAL USERS SHOULD KEEP ACCURATE EXPERIMENTAL RECORDS, AND BE ABLE TO PROVIDE AN AUDIT TRAIL OF THEIR ANIMAL EXPENDITURES AND USE THAT CORRELATES TO APPROVED PROTOCOLS.

****

PROTOCOL COVER SHEET: Requires a minimum of three signatures to include: the Primary Investigator, the individual responsible for scientific review and the Attending Veterinarian. In addition, the signature from the individual performing the statistical review on this cover sheet is recommended. If no signature block is present for a person who does the statistical review, then the following statement must be present on the protocol cover sheet. "A person knowledgeable in statistics has reviewed the experimental design." This Protocol Cover Sheet can also hold any additional information deemed necessary by the organization (Co- investigators, Department/Division Chief, Coordinating Departments, IACUC Chair, Biosafety Review etc.)

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:
(Signature Required) _____
(Principal Investigator)

SCIENTIFIC REVIEW: Signature verifies that this proposed animal use protocol has received appropriate peer scientific review, and is consistent with good scientific research practice. (No response is required to the title paragraph of this section)

(Signature Required) _____
(Research Unit Chief/Directors signature)

ATTENDING/CONSULTING VETERINARIAN: (Example) The attending/consulting veterinarian has reviewed the protocol and was consulted in the planning of procedures that require veterinary input, i.e., an unalleviated pain procedure. In addition, the veterinarian/veterinary medicine department has assisted with coordination for veterinary support to the protocol. (No response is required to the title paragraph of this section)
(Signature Required) _____
(Attending/Consulting Veterinarian)

STATISTICAL REVIEW: A person knowledgeable in statistics has reviewed the experimental design. (No response is required to the title paragraph of this section) (Inclusion of Signature Block is Recommended, but Optional)

_____
(Statistician)

OTHERS: You may wish to add specific additional offices or signature blocks for individuals responsible for coordination or compliance issues pertinent to your facility or operation. (i.e. Co-investigators, Coordinating Departments, IACUC Chair, Biosafety Review etc.)

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:

CO-INVESTIGATOR(S):

I. NON-TECHNICAL SYNOPSIS: A brief, narrative description of the proposal or idea that is easily understood by non-scientists.

II. BACKGROUND:

A. Background: This should include a brief statement of the requirement or need for the information being sought. Lengthy explanations are not required. Typically, the "literature or the experience that led to the proposal will be briefly reviewed" (AR 70-18), and a description of the general approach should be provided. Unnecessary duplication of effort should be strictly avoided.

B. Literature Search: This search must be performed to prevent unnecessary duplication of previous experiments. A search of Federal Research in Progress (FEDRIP) and DTIC databases or their equivalent is required for DOD funded research. An additional search of the scientific literature (MEDLINE, GRATEFUL MED, MEDLARS, AWIC, etc.) is highly recommended.

1. Literature Source(s) Searched:

2. Date and Number of Search:

3. Key Words of Search:

4. Results of Search: Provide a narrative description of the results of the literature search(s).

III. OBJECTIVE\HYPOTHESIS: In non-technical terms, state the objective of this protocol, or the hypothesis to be accepted or rejected.

IV. MILITARY RELEVANCE: With regards to military needs and mission requirements, this paragraph should provide a brief and succinct military justification for the research. If applicable state the Science and Technology Objective (STO) that this work supports.

V. MATERIALS AND METHODS:

A. Experimental Design and General Procedures: Provide a "complete description of the proposed use of animals." This section should succinctly outline the formal scientific plan and direction for experimentation. If several experiments or sequential studies

are to be included in the protocol, description of the experimental design for each separate experiment should be contained in sub-parts to this section. The length and detail required in this section depends largely on the complexity of the study. However, **a clearly understandable description of the numbers of animals and their distribution into experimental groups is essential.** The number requested should be the minimum numbers necessary to complete the study, but must be sufficient to yield meaningful results. If too few animals are requested and statistical significance is not achieved, the animals will have been misused. Be certain to include animals necessary for controls or technique development, etc. If the design is complex, a summary table or flow chart showing the distribution of animals by experimental group should be included. **The total number of animals required for the study is listed in section V.B.4. It is critical that reviewers of this protocol are able to follow your reasoning and calculations for the number of animals required, and can verify that the experimental design clearly supports the number of animals requested.**

1. Experiment 1:

2. Experiment 2: (etc.)

B. Laboratory Animals Required and Justification:

1. Non-animal Alternatives Considered: Were alternatives to animal use considered? **No study using animals should be considered prior to the elimination of all reasonable possibilities that the question might be adequately answered using other than animal means,** i.e., computer modeling, cell cultures, etc.

2. Animal Model and Species Justification: It is important that you adequately justify that animals are necessary for attainment of the research/training objectives. Moreover, justify the selection of this particular animal model. Investigators should use the least sentient species that will permit the attainment of research objectives. Why was this particular animal chosen? Were there other animal models considered that are lower on the phylogenetic scale (e.g., mice instead of rabbits)? Is there a unique quality or usefulness about this species that warrants its selection for use?

3. Laboratory Animals: No response necessary to the title paragraph of this section.

a. Genus & Species:

b. Strain/Stock: If inbred or specialized animals are required, please use proper terminology.

c. Source/Vendor: Provide a preferred source for the animals. Procurement of animals from non-USDA licensed sources requires an exception to policy. Enter the source/vendors USDA license number if available.

d. Age:

e. Weight:

f. Sex:

g. Special Considerations: Specialized requirements for the research animals should be reflected here, i.e., SIV or herpes antibody free, Pasteurella free, etc.

h. Other:

4. Total Number of Animals Required:

(a)	mice	320
(b)	guinea pigs	175

All that is required in this section is the total number of animals to be used on the study. The number requested here should match exactly those described in para V. A., Experimental Design & General Procedures in the MATERIALS AND METHODS section. Keep in mind the number requested should be the minimum numbers necessary to complete the study, but must be sufficient to yield meaningful results. If too few animals are requested and statistical significance is not achieved, the animals will have been misused. Be certain to include animals necessary for controls or technique development, etc. If additional animals are needed due to technical or unavoidable circumstances, or to exploit a serendipitous finding, follow IACUC procedures for requesting approval for additional animals.

5. Refinement, Reduction, Replacement: The DoD is often required to provide specific examples of its alternatives initiatives. Does this protocol have any provisions that would qualify it to be identified as one that refines, reduces or replaces (3 R's) the use of animals? For example, does your study use statistical tests that require fewer animals, i.e., a modified LD50 test like Thompson & Weil, or are you using cell cultures, computer modeling or any other technique that will influence the numbers of animals required? Are you using animals lower on the phylogenetic scale? Please provide a short description of the features that you feel qualify the study as one that employs one of the "3 R's," or give a negative reply. No response is needed under the title paragraph of this section.

a. Refinement: The use of analgesia, or the use of remote telemetry to increase the quality and quantity of data

gathered or adjusted early endpoint for the animals are examples of refinements.

b. Reduction: Use of shared control groups, preliminary screening in non-animal systems or innovative statistical packages are examples of reductions.

c. Replacement: Non-animal systems that eliminate the use of animals are examples of replacement.

C. Technical Methods: These should be presented in sufficient detail, documented or referenced, so that the IACUC can adequately review the procedure and obtain a clear understanding of what is to be done, how the animal will be handled, and make a reasonable determination as to whether this proposed use of laboratory animals is in compliance with DoD regulations, guidelines, and federal law. No response is needed under the title paragraph of this section.

1. Pain: The law defines a painful procedure as one that would "reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures." **If a procedure involves pain or distress, the P.I. must consult with the attending veterinarian.** Respond N\A if the animals will experience "no pain or distress."

a. USDA (Form 18-3) Pain category:

This information is reported by the organization to the USDA on USDA Form VS 18-23. **The P.I. or primary user should estimate the number of animals that will be counted in each pain category.** There are many situations where there are animals in more than one category, i.e., control animals. If more than one species is requested in the proposal, reflect those animals in a duplicate table in this paragraph. **The total numbers reflected in these three categories should add up to the number and percent of animals requested for the entire protocol in para V.B.4.**

(1) No Pain _____(#)_____% (Column C)

Studies involving no pain or distress beyond that expected on a momentary nature such as would occur with an injection, a deep palpation, grooming activities, etc.

(2) Alleviated Pain _____(#)_____% (Column D)

Procedures wherein anesthesia or analgesia will be administered to avoid or alleviate pain or distress. General anesthesia given for

surgical preparations, or the use of analgesia or anti-inflammatories would be examples for this category.

(3) Unalleviated Pain or Distress

_____(#)_____% (Column E)

Procedures where alleviation of pain or distress are contraindicated for some justifiable reason such as would confound the experimental results if drugs relieving pain were administered. Detailed justification for putting animals into this category is required below in para V.C.1.d.

b. Pain Alleviation: The attending veterinarian should be able to provide assistance in completing this section of the proposal.

(1) Anesthesia/Analgesia/Tranquilization: Describe the methods or strategies planned to alleviate pain or distress. If pain alleviation is planned, specify who will be administering the analgesics, anesthetics, or tranquilizers during the study. Provide agent, dosage, route & site, indication, needle size, etc.

(2) Paralytics: No use of paralytic agents without anesthesia is allowed unless scientifically justified by the P.I. and approved by the IACUC.

c. Alternatives to Painful Procedures:

(1) Source(s) Searched: e.g., AWIC, AGRICOLA, CAAT, MEDLINE, etc.

(2) Date of Search:

(3) Key Words of Search: e.g. Pain, surgery,

(4) Results of Search: Provide a narrative description of the results of the alternatives literature search. "Research facilities will be held responsible, if it is subsequently determined that an alternative to a painful procedure was available to accomplish the objectives of the proposed experiment." The Animal Welfare Act specifically states that the **"P.I. must provide a narrative description of the methods and sources, e.g., the Animal Welfare Information Center, MEDLINE, LIFE SCIENCES ABSTRACTS, AGRICOLA, AND BIOSIS that he\she used to determine that alternatives to the painful procedure were not available."** It is a requirement to perform the alternatives literature search and painful procedure justification even when animals are placed in the alleviated pain category (column D).

d. Painful Procedure Justification: Procedures causing more than transient or slight pain that are unalleviated, must be justified on a scientific basis in writing by the P.I. The pain must continue for only the necessary period of time dictated by the experiment, and then be alleviated, or the animal humanely euthanized. This paragraph must be completed if there are any animals listed in either the alleviated (column D) or the unalleviated pain or distress (column E) category in para V.C.1. **The P.I. must consult with the attending veterinarian or his or her designee in the planning of both alleviated and unalleviated painful procedures, and state it here.**

2. Prolonged Restraint: Describe and justify in detail any prolonged restraint (greater than twelve hours) intended for use during the study, e.g., primate chairs, restraint boards, metabolism cages, etc. Also describe habituation procedures for the prolonged restraint. This section is not intended for short-term actions such as rabbit restraint for bleeding, etc. If there is prolonged restraint involved, who will be restraining the animals, and for how long?

3. Surgery: Major operative procedures on non-rodent species, i.e., rabbits, monkeys, etc., should be conducted only in dedicated facilities intended for that purpose, and operated and maintained under aseptic conditions. Non-major operative procedures & all rodent surgery do not require a dedicated facility, but must be performed using aseptic technique, i.e., surgical gloves, mask, sterile instruments. A major operative procedure is one that "penetrates and exposes a body cavity, or causes permanent impairment of physical or physiological function." The animal care unit personnel should assist in defining the requirements of this portion of the law if necessary. No response required under the title paragraph of this section.

a. Procedure: Describe in detail any surgical procedures planned.

b. Pre- and Postoperative Provisions: Detail the provisions for both pre- and postoperative care, including provisions for post-surgical observations. Also include the provider of that care, and the location for the postoperative care.

c. Location: Give the location\room # for the proposed surgical procedure.

d. Multiple Survival Surgery Procedures: If multiple major operative procedures on the same animal are intended, they must be adequately justified for scientific reasons by the P.I. in writing.

(1) **Procedures:**

(2) **Scientific Justification:**

4. Animal Manipulations: Any injections, sampling procedures, or other manipulations of the animals necessary for the execution of the study must be described if not listed in section V. List needle sizes, routes of injection or withdrawal and anatomical location, e.g. 21 ga needle, SQ, IM, femoral vein, jugular vein etc., or the proposed method so that a reasonable evaluation of the appropriateness of the procedure can be made. You may furnish the committee a reference or SOP to document a particular procedure in lieu of a detailed description. You may wish to rearrange the subparagraphs of this section to suit your protocol. No response is needed under the title paragraph of this section.

a. Injections: There is no need to duplicate specific information already provided in section V.C.1.b., the Pain Alleviation, anesthesia/analgesia section of the proposal.

b. Biosamples: Cerebral taps, blood sampling, etc. List amounts taken and method for sampling. Procedures performed or biosamples obtained during a necropsy need not be described here.

c. Animal Identification: Microchip, tattoo, ear tags, cage cards, etc.

d. Behavioral Studies: Fully describe any intent to use aversive stimuli, food or water deprivation, etc, that would impact upon the animals in this study.

e. Other procedures: EKG's, radiology, aerosol exposure, etc.

5. Adjuvants: List any adjuvants and your plan for their use. Provide dosages & route.

6. Study Endpoint: What is the projected end point or termination of the study for the animals? Is death, euthanasia, or recovery expected; and what is the specific plan for determining when the animal experimentation phase will be stopped? You should ensure that unnecessary pain or distress is prevented by carefully considering "When is the experimental question answered?" so that the animals can be removed from the study as soon as feasible. Explain the plan for the disposition of surviving animals. **You must specifically address and justify any proposed use of death as an endpoint.**

7. Euthanasia: Explain the plan for euthanasia of the animals at the completion of the study and who will perform the procedure. The AWA defines euthanasia as "humane destruction of an animal by a method that produces rapid unconsciousness and subsequent

death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death." The current AVMA guidelines for euthanasia must be followed. Exceptions to the AVMA guidelines will be considered by the IACUC on a case-by-case basis. Exceptions must be scientifically justified by the P.I. in writing. The attending veterinarian will assist in selecting the best method for euthanasia if requested.

D. Veterinary Care: Attending veterinary care of lab animals receives particular emphasis in the AWA. The attending veterinarian of your facility will assist P.I.s with preparing this section if requested. No response is necessary to the title paragraph of this subsection.

1. Husbandry Considerations: The law specifically states that animal housing and living conditions must be appropriate to their species, and contribute to their health and comfort. Describe husbandry or refer to SOP. If known, list the location the animals will be routinely housed and the length of housing requirement. Personnel in the animal care unit should be able assist P.I.s in the preparation of the protocol sections dealing with animal care issues.

a. Study Room: If stay exceeds 12 hours.

b. Special Husbandry Provisions: Micro-isolators, metabolic cages, etc.

2. Attending Veterinary Care: Will the animals be observed daily or more frequently, and by whom? What is the plan if the animal becomes ill or debilitated during the study and requires supportive therapy? Will the animal be euthanized if it becomes critically ill or comatose, and by whom (study endpoint adjustment)? Justification for not providing supportive care for clinically ill animals is necessary.

3. Enrichment Strategy: Written justification for restricting enrichment programs or activity programs of dogs, cats, or nonhuman primates must be provided.

a. Dogs: Do you have any reason to restrict activity programs for dogs on this protocol that might be implemented by the animal care unit to comply with federal welfare regulations. If yes, justify.

b. Nonhuman Primates: Do you have any reason to prohibit environmental enrichment or enhancement strategies that might be implemented by the animal care unit to comply with federal welfare regulations. If yes, justify.

E. Data Analysis: List the statistical test(s) planned or the strategy intended to evaluate the data.

F. Investigator & Technician Qualifications/Training: List those animal procedures or manipulations described in the protocol that will be performed by each investigator or technician, and their training or qualifications to perform these procedures. Personnel conducting the "hands-on" animal procedures described in the protocol must be identified and appropriately trained and qualified to perform that procedure. **This is NOT questioning the P.I.'s PROFESSIONAL qualifications to conduct the research, but rather a requirement that personnel actually performing the research animal manipulations are technically competent, and thus are not inflicting unnecessary pain, distress, or injury to an experimental animal due to inexperience or improper technique.** Contact your attending veterinarian for assistance with this requirement.

VI. Biohazard/Safety: Provide a list of any potential biohazards associated with this proposal, e.g., viral agents, toxins, radioisotopes, oncogenic viruses, chemical carcinogens, etc. Explain any safety precautions or programs designed to protect personnel from biohazards, and any surveillance procedures in place to monitor potential exposures.

(Start new page here)

VII. ASSURANCES: The law specifically requires several written assurances from the P.I. It states that "research facilities will be held responsible if it is subsequently determined that an experiment is unnecessarily duplicative, and that a good faith review of available sources would have indicated as much."

(This section will state) As the Primary Investigator on this protocol I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a deviation is specifically approved by the IACUC.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used."

D. Biohazard\Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, etc., in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" which the DoD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible, and conducting humane and lawful research.

(Signature Required) _____

(Primary Investigator)

G. Painful Procedures: (Include only if conducting research that will cause more than slight or momentary pain or distress (Column D or E by USDA classification) the following statement must follow.) **I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals that WILL BE relieved or WILL NOT (circle one) be relieved with the use of anesthetics, analgesics and/or tranquilizers.** I have considered alternatives to such procedures; however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of the proposed experiment.

(Signature Required) _____

(Primary Investigator)

VIII. Enclosures: (Available for the attachment of the results of any literature searches, SOPs, references, or other documents pertinent to the protocol that you may wish to include. Local IACUC's should determine specific items to be included here.)

A. Literature Searches: DTIC, FEDRIP, MEDLINE, AGRICOLA, etc.

B. Pathology Addendum: Optional information

C. Pain Scoring Guidelines:

D. Adjuvant Policy:

PROTOCOL COVER SHEET

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:
(Signature Required) _____
(Principal Investigator)

SCIENTIFIC REVIEW:

(Signature Required) _____
(Research Unit Chief/Directors signature)

ATTENDING/CONSULTING VETERINARIAN:

(Signature Required) _____
(Attending/Consulting Veterinarian)

STATISTICAL REVIEW: A person knowledgeable in statistics has reviewed the experimental design. (No response is required to the title paragraph of this section) (Inclusion of Signature Block is Recommended, but Optional)

_____
(Statistician)

***OTHERS:**You may wish to add specific additional offices or signature blocks for individuals responsible for coordination or compliance issues pertinent to your facility or operation. (i.e. Co-investigators, Coordinating Departments, IACUC Chair, Biosafety Review etc.)

PROTOCOL TITLE:

PRINCIPAL INVESTIGATOR:

CO-INVESTIGATOR(S):

I. NON-TECHNICAL SYNOPSIS:
II. BACKGROUND:
- **A. Background:**
- **B. Literature Search:**
  1. **Literature Source(s) Searched:**
  2. **Date and Number of Search:**
  3. **Key Words of Search:**
  4. **Results of Search:**

III. OBJECTIVE\HYPOTHESIS:
IV. MILITARY RELEVANCE:
V. MATERIALS AND METHODS:
- **A. Experimental Design and General Procedures:**

B. Laboratory Animals Required and Justification:
   1. Non-animal Alternatives Considered:
   2. Animal Model and Species Justification:
   3. Laboratory Animals:
      a. Genus & Species:
      b. Strain/Stock:
      c. Source/Vendor:
      d. Age:
      e. Weight:
      f. Sex:
      g. Special Considerations:
      h. Other:
   4. Total Number of Animals Required:
   5. Refinement, Reduction, Replacement:
      a. Refinement:
      b. Reduction:
      c. Replacement:

C. Technical Methods:
   1. Pain:
      a. USDA (Form 18-3) Pain category:
         (1) No Pain _____(#)_____% (Column C)
         (2) Alleviated Pain _____(#)_____% (Column D)
         (3) Unalleviated Pain or Distress
             _____(#)_____% (Column E)
      b. Pain Alleviation:
         (1) Anesthesia/Analgesia/Tranquilization:
         (2) Paralytics:
      c. Alternatives to Painful Procedures:
         (1) Source(s) Searched:
         (2) Date of Search:
         (3) Key Words of Search:
         (4) Results of Search:
      d. Painful Procedure Justification:
   2. Prolonged Restraint:
   3. Surgery:
      a. Procedure:
      b. Pre- and Postoperative Provisions:
      c. Location:
      d. Multiple Survival Surgery Procedures:
         (1) Procedures:
         (2) Scientific Justification:
   4. Animal Manipulations:
      a. Injections:
      b. Biosamples:
      c. Animal Identification:
      d. Behavioral Studies:
      e. Other procedures:
   5. Adjuvants:
   6. Study Endpoint:

7. **Euthanasia:**

D. **Veterinary Care:**

1. **Husbandry Considerations:**

a. **Study Room:**

b. **Special Husbandry Provisions:**

2. **Attending Veterinary Care:**

3. **Enrichment Strategy:**

a. **Dogs:**

b. **Nonhuman Primates:**

E. **Data Analysis:**

F. **Investigator & Technician Qualifications/Training:**

VI. **Biohazard/Safety:**

(Start new page here)

VII. ASSURANCES: As the Primary Investigator on this protocol I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a deviation is specifically approved by the IACUC.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used."

D. Biohazard\Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, etc., in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" which the DoD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible, and conducting humane and lawful research.

(Signature Required) _____

(Primary Investigator)

G. Painful Procedures: (Include above if conducting research that will cause more than slight or momentary pain or distress (Column D or E by USDA classification) the following statement must follow.) **I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals that WILL BE relieved or WILL NOT (circle one) be relieved with the use of anesthetics, analgesics and/or tranquilizers.** I have considered alternatives to such procedures; however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of the proposed experiment.

(Signature Required) _____

(Primary Investigator)

VIII. Enclosures: (Available for the attachment of the results of any literature searches, SOPs, references, or other documents pertinent to the protocol that you may wish to include. Local IACUC's should determine specific items to be included here.)

A. Literature Searches: FEDRIP, DTIC, MEDLINE, AGRICOLA, etc.

B. Pathology Addendum: Optional information

C. Pain Scoring Guidelines:

D. Adjuvant Policy:

Appendix F

DoD Semiannual Program Review and Facility Inspection Checklist

DOD SEMIANNUAL PROGRAM REVIEW/FACILITY INSPECTION CHECKLIST-MANDATORY

Completion of this one-page checklist by the IACUC during the semi-annual program review and facility inspection is mandatory.

ORGANIZATION:_____ DATE OF REVIEW:_____

EVALUATION VIA CATEGORY	S	M	U	NA	EVALUATION VIA CATEGORY	S	M	U	NA
AAALAC History					**Identification Records**				
Administrative Commitment					**Emergency, Weekend & Holiday Care**				
Administrative Organization					**Adequate Veterinary Care**				
Institutional Policies					**Preventive Medicine**				
Animal Care & Use Committee					**Animal Procurement**				
Protocol Review Procedures					**Quarantine Isolation**				
Personnel Qualifications					**Control of Animal Disease**				
Personnel Hygiene					**Diagnostic Resource**				
Occupational Health Program					**Anesthesia & Analgesia**				
Animal Restraint					**Surgery & Postsurgical Care**				
Multiple Major Surgeries					**Euthanasia**				
Animal Husbandry					**Physical Plan Arrangement/Cond.**				
Housing/Caging & Pens					**Support Areas**				
Social Enrichment					**Cage Sanitation Fac.**				
Activity/Exercise					**Storage Facilities**				
Food/Water/Bedding					**Surgery Facilities**				
Sanitation					**Animal Rooms**				
Waste Disposal Methods					**HVAC**				
Vermin Control					**Emergency Power**				
Farm Facilities					**Animal Use Laboratories**				

KEY: S = Satisfactory; M = Minor Deficiency; U = Unsatisfactory/Major deficiency; NA = Not Applicable

USE OF CHECKLIST IN PROGRAM EVALUATION-- Completion of this one page checklist is mandatory. Any area that has minor or Major/Unsatisfactory deficiencies should be further explained on a separate page(s). Moreover, the listing of the minor or major deficiency should also include a plan of action for correction of the deficiency.

DETAILED OUTLINE OF CHECKLIST-- Utilization of this outline is optional. Attached is a detailed outline which follows this checklist. The outline includes most additional DoD requirements and is very similar to the program description outline used by organizations applying for AAALAC accreditation. This outline or one devised by your IACUC can be used to augment your semiannual program reviews.

USE OF ROOM INSPECTION FORM-- Utilization of attached form is optional. The use of this form or one developed by your organization may be useful in augmenting your semi-annual program review.

MINORITY OPINIONS-- Utilization of attached form is optional. All minority opinions must be included in the IACUC report. In addition it is mandatory that a majority of IACUC members sign the semi-annual report.

There **were** / **were not** (circle one) minority opinions in this semi-annual review.

-OPTIONAL-

DETAILED OUTLINE OF CHECKLIST-- Utilization of this outline is optional. Attached is a detailed outline which follows this checklist. The outline includes most additional DoD requirements and is very similar to the program description outline used by organizations applying for AAALAC accreditation. This outline or one devised by your IACUC can be used to augment your semiannual program reviews.

A. General Comments AAALAC history, administrative commitment, administrative organization,

B. Institutional Policies

1. Monitoring the Care and Use of Animals
   a. Institutional Animal Care and Use Committee
      1) Composition- New DoD Directive states the minimum number for IACUC membership is 5. New DoD policy states requires those IACUCS with only one non-affiliated member the IACUC to also appoint an additional alternate non-affiliated member. New DoD policy states specific training requirements for non-affiliated IACUC members (8 hours).
      2) Protocol review procedures- New DoD Directive and policies require use of DoD standard protocol format. New requirements include documentation of literature searches for DTIC, FEDRIP and other searches as required.
      3) Review of programs for Care and Use of Animals- New DoD policy encourages Commanders/Directors/CEO's of DoD laboratories to invest in training at all levels for those that use animals.
   b. USDA Report
2. Veterinary Care
   a. Intensity -
   b. Responsibilities of the Veterinarian(s) -
   c. Involvement in monitoring the care of animals -
   d. Involvement in monitoring use of animals -
3. Personnel Qualifications
   a. Animal resource Professional/Management/ Supervisory Personnel -
   b. Animal Care Personnel -
   c. Research Staff -
   d. Use of Hazardous Agents -
4. Personnel Hygiene
   a. Work clothing provided -
   b. Laundering of work clothing -
   c. Shower and change facilities -
   d. Eating, drinking, and smoking policies -
   e. Eating, drinking, and smoking facilities -
5. Occupational Health and Safety Program
   a. Content of program -
   b. Program oversight -
   c. Participation by staff -
   d. Training on zoonosis and personal hygiene -
6. Experimentation involving Hazardous Agents
7. Animal Restraint -
8. Multiple Major Surgical Procedures -

C. Laboratory Animal Husbandry

1. Housing
   a. Caging and pens -

b. Social enrichment -
c. Activity/exercise -
d. Micro- & Macroenvironments -

2. **Food**
   a. Type -
   b. Vendor quality control -
   c. Storage -
   d. Type of feeders -
   e. Institutional quality control -
3. **Bedding**
   a. Type -
   b. Appropriateness for how used -
   c. Storage facilities -
   d. Quality control -
4. **Water**
   a. Source - Satisfactory.
   b. Treatment - Satisfactory.
   c. Quality control procedures -
5. **Sanitation**
   a. Cage & pan litter changing -
   b. Portable cage sanitation
      1) Frequency -
      2) Procedures and agents -
      3) Monitoring and effectiveness -
   c. Pens, Stalls, etc. -
   d. Sanitation of feeding implements -
   e. Watering Implements
      1) Water Bottles -
      2) Automatic watering system -
   f. Sanitation of transport cages and vehicles -
   g. Room sanitation -
   h. Waste disposal methods -
   i. Vermin control -
6. **Animal Identification**
   a. Methods for identification of each species -
   b. Information of cage cards -
   c. Individual animal records -
7. **Provisions for Emergency, Weekend and Holiday Care**
   a. Qualifications of individuals providing care -
   b. Procedures performed -
   c. Monitoring of environmental systems -

D. **Veterinary Care**
   1. **Preventive Medicine**
      a. Animal procurement -
      b. Quarantine, Stabilization and Isolation -
         1) Receiving and initial evaluation procedures -
         2) Quarantine facilities
            a) For random source animals -
            b) For purpose bred animals -

3) Quarantine procedures -

c. Separation by species, source and health status -

2. Surveillance, Diagnosis, Treatment, and Control of Animal Disease

a. Program

1) Daily observation of animals -
2) Procedures for providing veterinary care -
3) Medical Records maintenance procedures -
4) Preventive medicine program for each species -
5) Animal Health monitoring -

b. Diagnostic Resources

1) Clinical Laboratory -
2) Necropsy/histology -
3) Radiology -
4) Use of available diagnostic resources including commercial laboratories -

3. Anesthesia and Analgesia

a. Agents used for each species -
b. Guidelines provided by the Veterinarian -
c. Monitoring the use of A & A -
d. Training and experience of personnel who perform anesthesia -
e. Safety procedures for use of explosive/flammable agents -
f. Waste anesthetic gas scavenging -

4. Survival Surgery and Postsurgical Care

a. Non-rodent mammalian species

1) Professional supervision -
2) Qualifications of persons performing the surgery -
3) Qualifications of surgical technicians -
4) Aseptic Techniques -
5) Postoperative care -
6) Maintenance of PO care records -

b. Rodent species - use of cap, mask, surgical scrub, sterilized instruments used, hair clipped, .
c. Non-survival surgeries -

E. Physical Plant

1. Overview of General Arrangement and Condition of Facility
2. Support Areas

a. Clean cage storage -
b. Storage Areas -
c. Waste disposal facilities -
d. Lounge area for animal care personnel -
e. Administrative space -
f. Cage sanitation facilities -

1) Interior surfaces -
2) Sanitation equipment -
3) Environmental conditions for personnel -

g. Surgery facilities

1) Areas for

a) Surgery -
b) Animal preparation -
c) Dressing rooms -
d) Surgeon preparation -
e) Postoperative care -

3. Animal Rooms
    a. Interior surfaces -
    b. Lighting - Satisfactory.
    c. HVAC -
4. Other Features
    a. Emergency power -
    b. Environmental monitoring
        1) Animal rooms air flow -
        2) Relative air pressures -
        3) Temperature -
        4) Humidity -
    c. Security -
5. Miscellaneous Animal Care and Use Equipment

F. Special Considerations
1. Genetics and Nomenclature -
2. Facilities and Procedures for Animal Research Involving Hazardous Agents -
3. Farm Animals -

G. Study Areas Visited -

H. Laboratories Visited -

-OPTIONAL-
USE OF ROOM INSPECTION FORM--Utilization of attached form is optional. The use of this form or one developed by your organization may be useful in augmenting your semi-annual program review.

Building _____

==========

ROOM_____ Animal Holding Area Lab Other

==========

ROOM_____ Animal Holding Area Lab Other

==========

ROOM_____ Animal Holding Area Lab Other

==========

ROOM_____ Animal Holding Area Lab Other

==========

GENERAL COMMENTS:

-OPTIONAL-
MINORITY OPINIONS-- Utilization of attached form is optional. All minority opinions must be included in the IACUC report. In addition, it is mandatory that a majority of IACUC members sign the semi-annual report. This form or one developed by your organization must be used to document that there were/were not minority opinions and that a majority of the IACUC members reviewed and signed the semiannual program review and facility inspection.

There **were** / **were not** (circle one) minority opinions in this semi-annual review.

SEMIANNUAL IACUC INSPECTION/PROGRAM REVIEW SIGNATURE SHEET

The Animal Welfare Act requires IACUCs to review and inspect laboratory animal care and use programs on a semiannual basis. This form facilitates compliance with the requirement that at least a majority of members of the IACUC sign the semiannual report, and have a opportunity to express a minority opinion to the report. Minority opinions should be appended to the report in writing.

IACUC MEMBER	SIGNATURE	DATE	MINORITY OPINION YES	MINORITY OPINION NO

Appendix G

U.S. Government Principles for Animal Use

Appendix

U.S. Government Principles for Animal Use

Interagency Research Animal Committee's

U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training

The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires *in vivo* experimentation with a wide variety of animal species. Whenever U.S. Government agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals, the following principles shall be considered; and whenever these agencies actually perform or sponsor such procedures, the responsible institutional official shall ensure that these principles are adhered to:

I. The transportation, care and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable Federal laws, guidelines, and policies.[1]

II. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.

III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered.

IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.

VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studies. In any case, veterinary care shall be provided as indicated.

VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their inservice training, including the proper and humane care and use of laboratory animals.

IX. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal research committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

[1] For guidance throughout these Principles the reader is referred to the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council.

Published in the *Federal Register*, May 20, 1985, Vol. 50, No. 97, by the Office of Science and Technology Policy

Appendix H

DoD Inspector General Recommendations on the Use of Animals in DoD Medical Research Facilities and Contract Research Facilities

Appendix H

DoD Inspector General Recommendations on the Use of Animals in DoD Medical Research Facilities and Contract Research Facilities

MEDICAL RESEARCH FACILITIES

Recommendation 1: The Director of Defense for Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should issue Department of Defense policy that requires every Department of Defense research facility to:

1. Support, and as necessary develop, animal care and use training programs, and encourage certification for all personnel involved in the care, use, and treatment of the animals; and

2. Develop a formal checklist to be used by the Institutional Animal Care and Use Committee when conducting its semiannual inspection. The published reports should document use of the checklist. All members of the Institutional Animal Care and Use Committee should sign the report that also includes a statement indicating there are or are not minority opinions.

Recommendation 2: The Director of Defense for Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs) and the General Counsel, Department of Defense, should provide clear Department of Defense guidance concerning the requirements and qualifications of the non-affiliated member of the Institutional Animal Care and Use Committee. The guidance should establish eligibility requirements, professional qualification, and characteristics for committee members, and set the minimum number of non-affiliated members desired.

Recommendation 3: The Director of Defense for Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should direct the Armed Services Biomedical Research Evaluation and Management Committee to develop a standardized, comprehensive Department of Defense research protocol request form and require its use by all Department of Defense research facilities.

Recommendation 4: The Director of Defense for Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should ensure each research facility commander is provided with information concerning the commendable practices identified by the inspection teams for consideration in their animal care and use program.

CONTRACT RESEARCH FACILITIES

Recommendation 1: The Director of Defense Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should issue Department of Defense policy that requires the Military Departments and the research facilities operated by the Office of the Secretary of Defense to complete the following tasks before awarding any contract or grant that involves research using any live animals:

1. All extramural research proposals using live animals should be reviewed by a veterinarian trained and knowledgeable about laboratory animal medicine to ensure compliance with all Federal laws, and Department of Defense regulations and guidelines concerning the care and use of animals.

2. To ensure the facility is complying with the requirements in the Animal Welfare Regulation, the Department of Defense funding agency should contact the United States Department of Agriculture to obtain copies of the most recent inspection reports for a facility under consideration for a contract or grant.

Recommendation 2: The Director of Defense Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should issue Department of Defense policy that requires the Military Departments and research facilities operated by the Office of the Secretary of Defense to perform the following tasks after a contract or grant that involves live animal use is awarded:

1. A veterinarian knowledgeable about laboratory animal medicine should conduct site visits to evaluate the animal care and use program at contract research facilities using non-human primates, marine mammals, dogs, or cats; conducting research deemed sensitive; or cited by the United States Department of Agriculture as a research facility under investigation. The policy should include the requirements for the initial site visit and the conditions for follow-on site visits.

2. To ensure continued compliance with the Animal Welfare Regulation, the Department of Defense funding agency should contact the United States Department of Agriculture on a routine basis to obtain a copy of the most recent annual inspection report for each facility with an active contract.

Recommendation 3: The Director of Defense Research and Engineering, in coordination with the Assistant Secretary of Defense (Health Affairs), should direct the Military Departments and the research facilities operated by the Office of the Secretary of Defense to require that all contractor proposals for research using live animals include all the information contained in the standardized Department of Defense protocol request format.

Appendix I

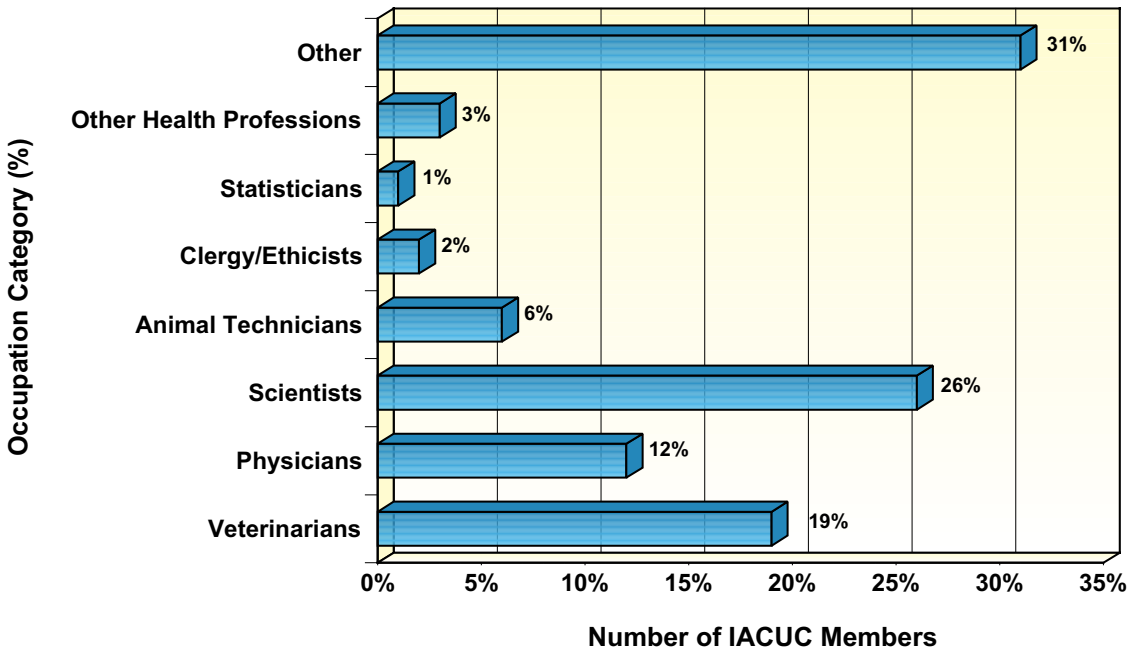
Occupations of Alternate IACUC Members (Voting and Nonvoting)

Appendix I

Occupations of Alternate IACUC Members (Voting and Nonvoting)

Alternate members often serve to provide backup expertise for the Institutional Animal Use and Care Committee (IACUC). Many are veterinarians, medical personnel or researchers. At some institutions, alternate positions are used to train new members toward assuming regular voting IACUC positions. While they vote only if regular members cannot attend an IACUC meeting, alternate members provide additional knowledge and experience to the panel and its deliberations. There were 79 alternate members in FY99.

Occupations of Alternate Members by Group



Occupations Categorized under "Other."

Administrator
Attorney
Business Administrator
Community Representative
Dance Instructor
Health Care Administrator
Instructor/Teacher
Logistician
Medical Construction Liaison
Personnel Manager
Program Analyst
Public Affairs Representative
Reference Librarian
Safety Representative
Secretary
Teacher

IACUC Membership Other Nonscientist Occupations

The following occupations are represented by one or more voting IACUC members: accountant (retired), attorney, aviator, dental technician, deputy commander, engineer, equipment manager, health care administrator, health system specialist, homemaker, hospital representative, learning specialist, librarian, logistician, personnel administrator, police officer, postal worker (retired), protocol review specialist, public affairs officer, Red Cross consultant, Red Cross volunteer, research administrator (nonscientific), resource strategy officer, safety engineer, salesman, secretary, signal core commander, social and health care worker, supply manager, teacher, and warehouse clerk.

Appendix J

Dissemination of Information on Animal Care and Use

Appendix J

Dissemination of Information on Animal Care and Use

- Posters throughout the facility advising employees and the public on procedures for filing animal care and use complaints emphasize that individuals do not have to use the chain of command but can go directly to the Institutional Animal Care and Use Committee (IACUC) chairman or the Inspector General (IG).
- Annual briefings to all facility personnel on the IG complaint process
- Notices posted on bulletin boards throughout the facility on how to register a complaint
- Mandatory investigator training courses
- Mandatory monthly seminars
- Researchers and technicians required to have documented appropriate training before performing procedures on animals
- Requirement for research staff and graduate students to complete training courses on the humane and ethical use of animals prior to engaging in research activities
- Provision of operating instructions and manuals to each investigator
- Posters announcing availability of anonymous "hot line" for registering concerns/complaints
- Provision of library resources, including books, manuals, and videotapes
- Provision of regulatory and policy documents
- Provision of journal and newsletter subscriptions
- Provision of investigators' procedural handbooks
- Briefings and veterinarian-directed discussions at IACUC meetings
- Provision of orientation training for new IACUC members

The following organizations provided courses and/or educational materials for the training of IACUC members, or conferences for their attendance, in FY99:

Scientists Center for Animal Welfare (SCAW)
American Association for Laboratory Animal Science (AALAS)
USDA Animal Welfare Information Center (AWIC)
Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)
Public Responsibility in Medicine and Research (PRM&R)
Applied Research Ethics National Association (ARENA)
National Association for Biomedical Research (NABR)
Laboratory Animal Welfare Training Exchange (LAWTE)

Examples of subjects listed in the context of training topics:

- Ethics and animal welfare
- Statistical analysis
- Distress minimization
- Reporting of deficiencies in animal care and use
- Handling and restraint
- Proper use of anesthetics
- Informational services and resources
- Procedural techniques
- Proper use of analgesics
- Security
- Surgical techniques
- Humane use of anesthetics and tranquilizers
- Legal and policy issues pertinent to animal care and use
- Animal husbandry
- Euthanasia
- Alternatives to animal use
- Animal models
- Prevention and control of zoonosis
- Alternatives to death as an endpoint
- Protocol training
- Occupational health and safety
- Minimizing animal use
- Literature searching strategies
- Public relations

Appendix K

Status of AAALAC Accreditation of DoD Animal Care and Use Facilities

Appendix K

Status of AAALAC Accreditation of DoD Animal Care and Use Facilities

I. U.S. DoD Facilities Accredited by AAALAC

I.1 OSD Components:

- Armed Forces Institute of Pathology, Washington, D.C.
- Armed Forces Radiobiology Research Institute, Bethesda, MD
- Uniformed Services University of the Health Sciences, Bethesda, MD

I.2 U.S. Army:

- U.S. Army Research Institute of Environmental Medicine, Natick, MA
- U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground
- U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD
- U.S. Army Center for Environmental Health Research, Fort Detrick, MD
- U.S. Army Soldier and Biological Chemical Command, Aberdeen Proving Ground, MD
- William Beaumont Army Medical Center, El Paso, TX
- Tripler Army Medical Center, Tripler Army Medical Command, Honolulu, HI
- Madigan Army Medical Center, Tacoma, WA
- U. S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD
- U.S. Army John F. Kennedy Special Warfare Center , Fort Bragg, Fayetteville, NC
- Walter Reed Army Institute of Research, Washington, D.C.
- Brooke Army Medical Center, Ft. Sam Houston, TX
- U.S. Army Medical Department Center and School, Ft. Sam Houston, TX
- Dwight David Eisenhower Medical Center, Fort Gordon, GA
- U.S. Army Dugway Proving Ground, Dugway, UT
- U.S. Army Institute of Surgical Research, Fort Sam Houston, TX

I.3 U.S. Navy:

- Naval Dental Research Institute, Naval Training Center, Great Lakes, IL
- Naval Medical Center, Clinical Investigation Program, San Diego, CA
- Naval Medical Center, Clinical Investigation and Research, Portsmouth, VA
- Naval Medical Research Institute, Bethesda, MD
- Space and Naval Warfare Systems Center, San Diego, CA

I.4 U.S. Air Force:

- Air Force Research Laboratory, Wright-Patterson AFB, OH
- Air Force Research Laboratory, Brooks Air Force Base, TX
- Keesler Medical Center, 81st Medical Group, Keesler AFB, MS
- Wilford Hall Medical Center, 59th Medical Wing, Lackland AFB, TX
- David Grant Medical Center, 60th Medical Group, Travis AFB, CA
- U.S. Air Force Academy, Colorado Springs, CO

II. Overseas Facilities Accredited by AAALAC:

- Naval Medical Research Institute Detachment, Lima, Peru
- Naval Medical Research Unit #2, Jakarta, Indonesia
- Naval Medical Research Unit #3, Cairo, Egypt
- Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand

Appendix L

The 1999 WRAIR DoD Laboratory Animal Care and Handling Workshop Schedule

Appendix L

The 1999 DoD Laboratory Animal Care and Handling Workshop Schedule

Division of Laboratory Animal Medicine
Walter Reed Army Institute of Research (WRAIR)

Issues in Laboratory Animal Care and Use Workshop

The Issues in Laboratory Animal Care and Use Workshop provides an overview of the WRAIR's laboratory animal program. The course focuses on ethical, regulatory, and humane considerations for writing and implementing animal use protocols, and will examine public and scientific concerns surrounding the use of animals in research. There is no laboratory portion associated with this workshop. However, it does include an optional 20-minute tour of the animal facility. The course is open to investigators, technicians, and administrative personnel. Class size is limited to 20.

Time: 0830-1230
Schedule: 29 January 1999
29 April 1999
10 June 1999
01 October 1999
28 January 2000

Nonhuman Primates & Safety Badge Class

The Nonhuman Primate and Safety Badge Class includes a didactic, safety, and lab portion focusing primarily on macaques. Individuals will take a short exam covering the special safety measures required for work with nonhuman primates. Upon passage of the exam, individuals will be issued a nonhuman primate room entrance authorization/medical alert badge. Further information about nonhuman primate safety issues or training in New World and other nonhuman primate species can be obtained by contacting the Division of Veterinary Medicine. Class size is limited to 10.

Time: 0830-1230
Schedule: 19 March 1999
30 April 1999
31 May 1999
16 July 1999
08 October 1999
03 December 1999
21 January 2000

Rodent Class (Rats, Mice, Guinea Pigs)

The Rodent Class is a general species specific course that includes both didactic and lab portions. Class size is limited to 10.

Time: 0830-1300
Schedule: 26 February 1999
02 April 1999
06 May 1999
04 June 1999
13 August 1999
03 September 1999
05 November 1999
10 December 1999

Lagomorph Class

The Lagomorph Class is a general species specific course that includes both didactic and lab portions. Class size is limited to 10.

Time: 0830-1200
Schedule: 05 February 1999
16 April 1999
11 June 1999
23 July 1999
10 September 1999
19 November 1999
21 January 2000

Swine Class

The Swine Class is a general species specific course that includes both didactic and lab portions. Class size is limited to 10.

Time: 0800-1300
Schedule: 05 March 1999
21 May 1999
17 September 1999
18 November 1999

Aseptic and Sterile Techniques Class

The Aseptic and Sterile Techniques workshop reviews the principles of aseptic and sterile techniques required by Federal law to support rodent survival surgeries. Survival surgeries involving animals other than rodents must be performed in a dedicated surgery. The class includes both didactic and lab sections. Live animals are not used. The workshop is open to investigators and technicians. Class size is limited to 12.

Time: 0830-1230
Schedule: 23 April 1999
22 July 1999
29 October 1999
09 December 1999

Introduction to Laboratory Animals Workshop

This course provides a broad overview of laboratory animal care and use policies, practices, and procedures, and includes a portion involving the "hands on" handling of rodents and rabbits. It does not involve instruction in research techniques and DoD workshop certificates are not given to attendees. Students actively involved in research must also take the regular DoD workshop for that species. Class is designed for high school students and college students who have never worked in a laboratory environment before. Upper level college students, and students with previous experience may take this class if they wish, but should also take the regular workshops.

Schedule:	25 June 1999	**Time:**	830-1200
	8 July 1999		830-1200
	9 July 1999		1230-1600

Appendix M

IACUC Training and Information

Appendix M

IACUC Training and Information

Nonaffiliated IACUC Member Training Recommendations

The following are some examples of topics and resources which would fulfill the congressionally mandated 8-hour training requirement for any new nonaffiliated IACUC members. The following were drawn from activities offered to nonaffiliated IACUC members.

Topics:	Resources:
1. Humane Care and Ethics Issues Dealing with Animal Use (This block should be no longer than 4 hours)	- Video (40 minutes) "IACUC Functions and the Humane Care and Use of Animals" available from the Laboratory Animal Training Association (LATA) - Questions and answers with the attending veterinarian - USAMRIID slide set (~200 slides covering Surgery, Euthanasia, Ethics, Pain and Distress) - Education and Training in the Care and Use of Laboratory Animals (National Academy Press, 1991)
2. Regulatory Responsibilities and Protocol Review Techniques (This block should be no longer than 4 hours)	- Overview of DoD protocol format with the attending veterinarian - Laboratory animal protocol review articles (available from the editor as a bound notebook with 2 years of reviews) - USAMRIID slide set covering responsibilities, laws and regulations (~100 slides)
3. Facility Familiarization Tour	- Attending veterinarian, facility manager, IACUC members
4. Basic Husbandry and Techniques of Laboratory Animals	- LATA video tapes and script - ACLAM slide sets with audio cassettes - USAMRIID slide set
5. Documentation of Training	- Each institute will develop a checklist and sign-in logo to verify training received

Additionally, we recommend the individual institute supplement in-house training programs by sending IACUC members to outside meetings such as Public Responsibility in Medicine and Research (PRIM&R)/Applied Research Ethics, National Association (ARENA) and American Association of Laboratory Animal Science.

Examples of Training and Information Provided to IACUC Members in FY99

Reading and discussion of animal welfare regulations and policies including: Discussion of *Guide for the Care and Use of Laboratory Animals*, Animal Welfare Act Regulations, DoD Directive 3216.1, AFI 40-401, and the DoD Protocol Format.

Viewing of video tapes "The Humane Care and Use of Laboratory Animals" (LATA, 1991) or a commercial tape, "Animal Care Matters."

Attendance at a veterinarian-developed course addressing ethics and animal welfare, animal care and use protocol requirements, humane methods for the care and handling of lab animals, veterinary care, zoonosis, proper use of anesthesia, analgesics and tranquilizers, aseptic surgical techniques, occupational health & safety, security, and public relations. Another institution-mandated course addressing: IACUC membership, role of the IACUC/protocol review, facilities inspection, role of the nonaffiliated member, role of the chairperson, laws and policies governing animal research (federal, state, military), the protocol review process, animal rights versus animal welfare, whistle blower protection, and reporting animal abuse.

Sending committee members to an outside training program that includes, at a minimum, 4 hours addressing regulatory issues and proper techniques on protocol review procedures and processing, and 4 hours addressing humane care and ethics dealing with animal use.

Distribution of training materials including: Laboratory Operating Instructions, procedures for reporting suspected animal misuse/abuse, the *Guide for Care and Use of Laboratory Animals*, the Animal Welfare Act, Animal Welfare Regulations, DoD Directive 3216.1 and Air Force Joint Instruction 40-401.

Development of a mandatory CD-ROM computer-based interactive training course for animal care personnel and IACUC members.

Informal training through monthly discussions of journal articles from laboratory journals such as: *Lab Animal*, *Journal of the American Veterinary Medical Association*, *Journal of Wildlife Diseases*, *Journal of Zoo and Wildlife Medicine*, and the *American Journal of Veterinary Research*. Elsewhere, IACUC members reviewed and discussed the journals of SCAW, *Contemporary Topics in Lab Animal Science*, *Laboratory Animal Science*, and *Lab Animal*, and a third IACUC was provided with monthly IACUC meeting packets containing articles from animal science journals and handouts from organizations such as AWIC, SCAW, NABR, AMP.

Nonaffiliated members attended meetings such as the Annual National SCAW meeting, or the ARENA/ PRIM&R conference where they took a 1-day course entitled "IACUC 101."

IACUC members were required to prepare verbal and written trip reports to share regarding their attendance at conferences organized by AALAS, PRIM&R, SCAW, LAWTE, and AAALAC.

Provision of a librarian-provided in-service to the IACUC on searching the literature for duplication of effort and identification of animal use alternatives

Members attended a SCAW 2-day seminar held in San Antonio, Texas entitled "IACUC's and Animal Well-Being: Responsibilities, Relationships, and Training."

Provision of the *Guide for Laboratory Animal Care and Use* to each IACUC member.

Abbreviations: USDA Animal Welfare Information Center (AWIC); Scientists Center for Animal Welfare (SCAW); National Association for Biomedical Research (NABR), Americans for Medical Progress (AMP); American Association for Laboratory Animal Science (AALAS); Public Responsibility in Medicine and Research (PRIM&R); Laboratory Animal Welfare Training Exchange (LAWTE), and Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC); Applied Research Ethics National Association (ARENA).

Appendix N

Animal Use Categories

Appendix N

Animal Use Categories

MEDICAL (M)

M1: Military Dentistry

Includes studies in the areas of:

- dental disease and management of dental emergencies
- testing medical devices for maxillofacial injury
- testing materials for maxillofacial injury
- surgical management of maxillofacial injury

M2: Infectious Diseases

Includes studies in the areas of:

- emerging infectious diseases of military importance
- vaccine development for prevention of bacterial sepsis and septic shock
- shigella vaccines
- malaria vaccines
- gonococcal peptide vaccine
- enterotoxigenic *E. coli* (ETEC) vaccine
- rickettsial diseases
- group A streptococcal vaccines
- polyvalent meningococcal vaccine
- prevention of Campylobacter diarrheal disease
- hepatitis virus vaccines
- establishment of diagnostic tests for infectious disease agents
- diagnosis of leishmaniasis
- development of drug therapies for infectious disease agents
- dengue virus vaccines
- viral hemorrhagic fever and encephalitis prevention and countermeasures
- identification and control of insect vectors of infectious diseases
- prevention of military HIV infection

M3: Medical Chemical Defense

Includes studies in the development of:

- medical countermeasures for vesicant agents
- medical pretreatment for cyanide
- prophylactic therapeutics for chemical agents
- reactive topical skin protectant
- medical countermeasures for respiratory agents
- chemical casualty management strategies and treatments

M4: Medical Biological Defense

Includes studies in the development of medical countermeasures for:

- *Yersinia pestis*
- brucellosis
- anthrax
- *Clostridium perfringens*
- Q-fever
- *Francisella tularensis*
- encephalomyelitis viruses
- variola
- Filoviridae
- physiologically active compounds
- sodium channel neurotoxins
- ricin
- staphylococcal enterotoxin B
- botulinum toxin
- venoms

M5: Human Systems Technology

Includes studies on:

- bioeffects of lasers
- laser impacts on performance
- treatment of laser-induced injury
- development of predictive models for a non-auditory exposure standard for blast overpressure
- development of occupational health protection criteria and exposure assessment technologies for toxic hazards arising from weapon systems and combat operations
- vibration
- bioeffects of electromagnetic radiation
- development of countermeasures for the effects of operational stress on military performance
- environmental injury
- development of methods, criteria, and predictive models for the risk of pulmonary injury in defeated armor scenarios

M6: Combat Casualty Care

Includes studies in:

- blood loss
- resuscitation
- secondary damage after hemorrhage
- soft tissue injury
- musculoskeletal injury
- combat stress injury
- burn injury
- anesthetics
- delivery systems

M7: Ionizing Radiation

Includes studies on:

- development of radioprotective compounds
- therapies for radiation-induced pathology
- bioeffects of ionizing radiation
- psychomotor effects of ionizing radiation
- mechanisms of radiation-induced pathophysiology

M8: Other Medical RDT&E

Includes studies in the areas of:

- breast cancer research
- neurofibromatosis research
- Gulf War illnesses
- laser research
- toxicology
- zoonosis
- free electron laser
- defense women's health research
- occupational medicine
- osteoporosis
- vectorborne diseases
- prostate cancer research
- ovarian cancer research
- environmental safety
- neurotoxin research
- bone health research
- disaster relief and emergency medical services

NON-MEDICAL (N)

N1: Physical Protection

As previously indicated, excludes reporting military working animals and includes:

- developing hearing protection criteria
- mechanisms of and protection from military acoustic hazards
- ocular effects and performance of eye protective devices

N2: Physical Detection

Includes studies in the development of:

- biosensors
- chemical detection devices
- the Chemical Biological Mass Spectrometer (CBMS) detector
- auditory detection thresholds in marine mammals
- models of dolphin echolocation
- detection of biological warfare agents

N3: Offensive Weapons Testing

No studies were performed in this category in FY99.

N4: Other Non-Medical RDT&E

Includes studies in the areas of:

- telemedicine
- bioengineering
- controlled biological systems
- environmental assessment
- environmental research
- fish in marine and freshwater aquaria
- hearing testing
- improving learning and decision making
- jet fuel toxicity
- jet lag and wakefulness
- marine biology
- neural interface systems using rodent models
- neural science
- physical detection
- physiology
- minimally invasive therapy
- sonar
- toxicology
- underwater propulsion

CLINICAL INVESTIGATIONS (C):

C1: Clinical Medicine

Research conducted includes a wide variety of clinical medical diseases/conditions that are not necessarily unique to the military.

Includes studies in the areas of:

- burn treatment
- prophylaxis against toxic chemicals
- wound healing
- preservation of tissue sample morphology
- differentiation of brain tumors
- substances promoting repair of sound-sensing cells
- regulation of tracheal mucin secretion by retinoic acid
- breast cancer research
- mechanisms and treatment of renal pathophysiology
- effects of tumor necrosis factor on gonadotrophic activity
- treatment of immune-mediated hearing loss
- mechanisms of lung growth and compensation following injury
- testing of hepatitis-E vaccines

C2: Clinical Surgery

Includes studies in the areas of:

- adverse effects on wound healing of post-surgical treatments
- development of synthetic materials for surgical closures
- topical stimulants of skin healing following biopsies
- techniques of fiberoptic bronchoscopy
- laparoscopic cholecystectomy
- biomechanical and histological effects of artificial implants
- identification and development of improved implant materials
- evaluation of new techniques to remove seminal vesicle cysts
- electrohydraulic lithotripsy

C3: Other Clinical Investigations

- anesthetic response study
- microgravity induced musculoskeletal pathology

TRAINING AND INSTRUCTIONAL (T):

T1: Training, Education, and/or Instruction for Personnel

Types of training include:

- animal technician training
- training of special forces medics
- investigator training in proper techniques used with animals
- physician training in medical or surgical procedures, etc.

The training locations included DoD laboratories or medical centers. Does not include experimental or research-related work.

T2: Other Training/Instruction

No studies were performed in this category in FY99.

ADJUNCTS AND ALTERNATIVES TO ANIMAL STUDIES (A):

A1: Adjuncts to Animal Use Research

Addresses those studies and uses that focus specifically on animal husbandry and care issues, and not directly on human medical, nonmedical, or training issues.

A2: Alternatives to Animal Investigation

Includes studies involving the use of animals that are designed to address directly and specifically issues of replacement, reduction, or refinement options for which animals are currently used; this classification does not include studies that are specifically directed at military RDT&E, clinical studies, or training requirements that may employ the animal alternatives of replacement, reduction, or refinement in the performance of the required protocols.

A3: Other Alternatives/Adjuncts

No studies were performed in this category in FY99.

CLASSIFIED SECRET OR ABOVE STUDIES (S):

S: Animals in Studies Classified SECRET or Above

Includes studies in which the information concerning the study may not be released for public knowledge because of the impact on national security.

ANIMAL BREEDING STOCK (B):

B: Animals Maintained for Breeding

Includes:

- animals maintained at the facility or supported through contract funds for breeding purposes to supply offspring to be used in animal-based research for particular work units or protocols
- breeding animals and offspring not assigned to specific work units or protocols

OTHER ANIMAL USE CATEGORIES (O):

O: Other Animal Use Purposes

Includes:

- animals awaiting assignment to protocols
- basic sleep research
- neurosciences
- quality assurance

Appendix O

Summary of Animal Use Data by Category

Appendix O

Summary of Animal Use Data by Category

A1

Animals Reported	Animals Used
Guinea Pig	62
Monkey	4
Mouse	330
Rat	173
Total A1	569

A2

Animals Reported	Animals Used
Fish	11,270
Frog	88
Total A2	11,358

B

Animals Reported	Animals Used
Mouse	2,000
Sand Rat	185
Total B	2,185

C1

Animals Reported	Animals Used
Chinchilla	53
Dog	33
Ferret	60
Goat	11
Guinea Pig	94
Hamster	5
Monkey	28
Mouse	7,907
Pig	134
Rabbit	181
Rat	3,042
Sheep	26
Sand Rat	73
Chicken	368
Total C1	12,015

C2

Animals Reported	Animals Used
Chinchilla	18
Dog	47
Ferret	43
Goat	130
Monkey	85
Mouse	91
Pig	294
Rabbit	442
Rat	1,002
Sheep	64
Total C2	2,216

C3

Animals Reported	Animals Used
Mouse	106
Rat	57
Total C3	163

M2

Animals Reported	Animals Used
Bird	66
Cat	7
Chicken	845
Cow	136
Dog	70
Duck	12
Ferret	27
Gerbil	42
Goat	36
Goose	36
Guinea Pig	658
Hamster	1,043
Horse	3
Jird	12
Monkey	450
Mouse	106,793
Rabbit	328
Rat	2,313
Sheep	93
Shrew	87
Weasel	1
Swan	5
Nonhuman Primate	533
Total M2	113,596

M3

Animals Reported	Animals Used
Guinea Pig	3,162
Monkey	56
Mouse	4,052
Pig	169
Rabbit	796
Rat	2,811
Chicken	20
Frog	22
Squirrel	32
Nonhuman Primate	7
Total M3	11,127

M4

Animals Reported	Animals Used
Burro	2
Cow	1
Goat	12
Goose	17
Guinea Pig	2,650
Hamster	954
Horse	3
Monkey	204
Mouse	45,792
Pig	25
Rabbit	28
Rat	605
Sheep	33
Nonhuman Primate	24
Fish	2,000
Total M4	52,350

M5

Animals Reported	Animals Used
Frog	102
Hamster	779
Monkey	59
Mouse	1,302
Pig	116
Rabbit	225
Rat	934
Total M5	3,517

M6

Animals Reported	Animals Used
Goat	72
Guinea Pig	34
Mouse	1,532
Pig	1,138
Rabbit	977
Rat	5,506
Calf	14
Dog	101
Nonhuman Primate	18
Sheep	1
Total M6	9,393

M7

Animals Reported	Animals Used
Dog	42
Guinea Pig	292
Rat	66
Total M7	400

M8

Animals Reported	Animals Used
Chipmunk	1
Frog	1,167
Guinea Pig	162
Hamster	4
Monkey	110
Mouse	67,117
Pig	28
Rabbit	290
Raccoon	3
Rat	9,937
Vole	1
Bushy-Tailed Woodrat	3
Cotton Mice	2
Deer Mice	701
Great Basin Pocket Mouse	3

M8 (cont.)

Animals Reported	Animals Used
Hispid Cotton Rat	3
Meadow Jumping Mouse	1
Meadow Vole	7
Mexican Woodrat	18
Northern Grasshopper	1
Ords Kangaroo Mouse	3
Pigeon	24
Pinyon Mice	45
Prairie Vole	2
Shorttail Shrew	1
Western Harvest Mouse	24
White-Footed Mouse	182
Calf	2
Chicken	2,400
Chinchilla	37
Dog	98
Nonhuman Primate	68
Sheep	4
Total M8	82,449

N1

Animals Reported	Animals Used
Deer	12
Monkey	110
Mouse	620
Rabbit	16
Rat	336
Total N1	1,094

N2

Animals Reported	Animals Used
Goat	9
Mouse	402
Rabbit	6
Rat	78
Snake	139
Total N2	634

N4

Animals Reported	Animals Used
Cow	2
Dolphin	38
Ferret	12
Fish	8,370
Frog	2,700
Goat	15
Mouse	3,265
Rabbit	276
Rat	2,579
Sea Lion	4
Sheep	9
Vole	30
Whale	3
Bobwhite Quail	263
Bat	4
California Sea Lion	2
Octodon Degu	12
Harbor Seal	1
North Elephant Seal	1
Dog	51
Nonhuman Primate	7
Pig	150
Total N4	17,794

O

Animals Reported	Animals Used
Guinea Pig	4
Mouse	300
Rat	216
Total O	520

S

Animals Reported	Animals Used
Goat	28
Monkey	47
Pig	30
Rat	166
Total S	271

T1

Animals Reported	Animals Used
Cat	21
Chicken	24
Dog	48
Ferret	116
Frog	50
Gerbil	3
Goat	2,194
Guinea Pig	195
Hamster	14
Monkey	67
Mouse	693
Pig	776
Rabbit	116
Rat	1,059
Sheep	50
Vole	20
Total T1	5,446

Grand Total Animal Use/Research	**327,097**

Appendix P

Specific Alternatives Implemented in FY99

Appendix P

Specific Alternatives Implemented in FY99

Replacement - The replacement alternative addresses supplanting animal use with nonliving systems, analytical assays, cell-culture systems, and with animals that are lower on the phylogenetic scale. Additionally, human subjects are used when experimental drugs and other procedures progress to human trials. Such trials are conducted in accordance with Title 32, U.S. Code of Federal Regulations, Section 219, "Protection of Human Subjects in DoD-Sponsored Research."

Alternative Subtype	Research Category	Alternative Description
Biochemical/physical methods	C1	Molecular biological techniques that are used to assess vasopressin receptor subtypes replace the need to do all characterization of hormone receptor sub-classifications with pharmacological tools in the whole animal.
	M4	Equine encephalomyelitis virus vaccine testing cell culture assays for determining the neutralization titers of sera replace the requirement for animals.
	M4	Cell culture growth of antibody-producing hybridomas in hollow fiber units replaces the requirement for mice to make ascites for use in the passive transfer studies.
	M4	In the risk assessment and evaluation of viral agents, vector cell cultures rather than suckling mice, are used as the isolation method of choice.
Non-mammalian species or species lower on the phylogenetic scale	M4	In equine encephalomyelitis virus challenge studies in rodents, non-animal systems, such as cell culture for virus titrations and plaque-reduction neutralization tests, substantially eliminate the use of animals for these determinations. Utilizing these rodent models for initial studies replaces more evolved animals such as rabbits and NHPs for selection of vaccine candidates.
	M4	The use of BALB/c mice represents replacement with animal species lower on the phylogenetic tree than those used in previous Ebola research experiments.
	M4	The use of mice for the study of antiviral drugs and viral pathogenesis replaces the use of NHPs for this purpose.
Other species replace companion animals (dogs and cats)	C2	Pigs have replaced the use of dogs in cardiovascular hypothermia studies.
	T1	Ferret has replaced the cat as the animal model in intubation training.
	T1	Pigs and ferrets have replaced dogs and cats in the Bronchoesophagology Course.
Replacement using in vitro cell cultures	C1	In HIV-1 fusion co-factors, tissue culture systems replace the use of mice for ascites production.
	M5	Pathophysical mechanisms of thermal injuries/illnesses studies use cell cultures using human blood exposed to heat stress.

Alternative Subtype	Research Category	Alternative Description
	M7	In anthrax vaccine studies, bacterial cultures are used to quantitate spore numbers thus reducing the number of rodents used.
	M7	In radiation injury treatment research, cell culture studies are used to identify drug action mechanisms and screen candidate compounds.
	M7	Screening experiments or optimizing radiation dose response assays were carried out in in vitro cell lines.
Replacement with computer simulation, models, or other technologies	A1	In the protocols for techniques in animal care and use, non-animal training aids were used to teach and supplement teaching of animal procedures.
	M4	Reference materials and non-animal alternatives, such as the Koken rat, are used as training aids.
Utilization of alternative biological testing methods	C2	Use of cadaveric joint specimens for development and refinement of Telemetric Fixation Device Transducer to measure anterior cruatiate ligament graft forces has replaced device development in live animals.
	N2	Monoclonal antibody production using phage display technology requires no mice for the production of antibody. Traditional monoclonal antibody production may require up to 20 mice/protocol for ascites production.

Reduction - Decreasing the number of animals used through the use of statistical or innovative design strategies, while preserving the scientific integrity of the biological model, is a major emphasis of the reduction alternative to animal use.

Alternative Subtype	Research Category	Alternative Description
Enhanced protocol design	B	In mouse and guinea pig breeding colonies, males and females are paired only when there is a projected need for offspring for use in experiments, reducing the number of animals that might be needlessly culled.
	C1	Gastric biopsies are used for multiple tests, reducing the number of biopsies taken, as well as the number of monkeys used.
	C2	By placing multiple implants in each knee, the total number of goats needed was reduced.
	C2	Monkeys serve as donor and recipient of kidney transplant for a tolerance induction study thereby reducing animal numbers by 50%.
	T1	ECMO clinical training course reduces the number of pigs by increasing the number of students training on each pig.
	T1	Surgical skills practicum for the advanced trauma life support course reduces the number of goats by increasing the number of students training on each goats and by the sharing of goats with other protocols.

Alternative Subtype	Research Category	Alternative Description
Substitution of computer simulation, models, or other technologies	C1	In the purification of the Streptococcal Hypertensive Compound, rats are used for screening purposes, thus greatly reducing the number of lambs needed.
	C2	In the evaluation of Laryngeal Mask Airways, photographs are used for analysis, which reduces the number of ferrets needed.
	T1	In microsurgery training, the use of multimedia procedural instruction and of non-animate training aids and models reduces the number of animals necessary for the training laboratory.
	T1	In the Advanced/combat trauma intervention training, the majority of training exercises are conducted using inanimate training models, such as mannequins and/or cadaver parts.
	T1	Training with plastic models or mannequins prior to the use of live animals reduces the number of animals used.
Utilization of alternative biological testing methods	M4	Iterations of the experiments on nucleic acid vaccines for tick-borne encephalitis viruses and vaccinia virus will be combined when possible to reduce the number of control animals used.
	T1	Use of non-animal training systems such as pelvic trainers, suturing practice models, and laproscopic trainers reduces the number of live animals required for the General Surgery Training laboratory.
	M4	In the last 2 years, the use of hollow fiber filters has successfully reduced mouse requirements by at least 50 mice per year in a study on the production of murine hybridomas.
	N4	Use of an isolated perfused liver preparation significantly reduces the number of animals required to conduct predictive toxicological studies.

Refinement - The refinement alternative for animal use addresses the need to ensure that the maximum humane use of each animal is obtained through proper protocol design and efficient utilization of animals, or through the modification of the experimental design to reduce the ethical cost associated with the study.

Alternative Subtype	Research Category	Alternative Description
Environmental enrichment	A1	Social and non-social enrichment provided for housed animals.
	C2	Ferrets were housed in group housing with multiple housing accessories for exploration and exercise.
	C2	Group housing with in-cage diversionary accessories is used for environmental enrichment.
	M2	Balls and gnawing treat (Bunny blocks) are provided in the rabbit cages for their environmental enrichment.

Alternative Subtype	Research Category	Alternative Description
	M2	The geese are allowed to have "swimming hours" once a week in a temporary pond created for them. Perch is added to the pond to simulate dry ground during the swimming hours.
Reduce distress	M5	Minimum number of animals used and all animals given chew toys, balls, and group housing for environmental enrichment.
	B	Breeding pairs will be kept together throughout their lives which will significantly reduce stress on animals.
	B	Use of natural breeding methods.
	C1	Blood and urine samples are obtained via indwelling catheters, which eliminates the need for repeated invasive manipulations of the animal to collect the samples.
	C1	The use of long-term in-dwelling catheters with a subcutaneous port for cerebrospinal fluid access, and a carotid loop for arterial access allows biosamples to be obtained with minimal distress to the animals.
	C2	Unilateral rear limb surgery was used in this protocol which reduces stress from the use of both rear limbs. Use of both rear legs requires prolonged restraint.
	M2	Elimination of footpad injections to test for delayed type hypersensitivity.
	M2	Adaptation of several mosquito colonies to membrane feeding so that animals are not exposed directly to mosquitoes.
	M4	The use of implanted osmotic pumps avoids repeated medication dosing and minimizes animal handling and distress.
Reduce pain	M7	Telemetry is used to collect body temperature data.
	T1	Instructors use mannequins to orient students to anatomy and emergency procedures prior to the live animal lab.
	C2	Use of radiography and ultrasonography replaces the need for multiple survival surgeries to evaluate the progress of the animal subject.
	M2	The 100% infectious dose (the dose of bacterial cells that causes 100% infection in mice as measured by a variety of clinical signs) will be used instead of the 50% lethal dose which measures only mortality.
	M4	The use of whole-body plethysmography to measure individual minute volume and breathing rate to determine accurately the inhaled dose for each monkey will allow the minimum number of animals to be used to obtain statistically significant results.
	N4	Surface and subdermal electrodes are used which are more humane than in-dwelling electrodes.

Appendix Q

Food and Drug Administration Group Recognition Award

Food and Drug Administration

Group Recognition Award

PRESENTED TO

Harry Salen

as a member of Interagency Regulatory Alternatives Group (IRAG)

For outstanding contribution in facilitating the reduction, refinement and replacement of animal testing, and advancing the development of non-whole animal alternative methods.

May 9, 1997

DATE

Michael A. Friedman

LEAD DEPUTY COMMISSIONER
FOOD AND DRUG ADMINISTRATION